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Studies in the Family Orobanchaceae. I. A Contribution to the Embryology of Cistanche tubulosa Wight.

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INTRODUCTION

The Orobanchaceae comprise leafless, brown or yellowish white root parasites. The most important genus, *Orobanche,* occurs as a serious parasite on some field and garden plants of the families Solanaceae and Cruciferae. In some countries a species of *Aeginetia, A. indica,* causes considerable damage to the sugarcane crop.

Studies on the morphology of the Orobanchaceae by Schacht (1850), Caspary (1854), Koch (1876–87), Heinricher (1896), Bernard (1903),[1] and Worsdell (1897), are mostly fragmentary. Worsdell described the development of the ovule in *Christisonia neilgherrica.* But his study of the endosperm and haustoria is superficial and in some ways he seems to have been misled by the inaccurate observations of his predecessors. Persidsky (1926) studied the embryology of *Orobanche cumana* and *O. ramosa* and Carter (1928) made a cytological study of the gametophytes of *O. minor.* The most detailed account is by Glišić (1929) who made a thorough study of the development of the embryo sac and endosperm in *O. hederae* and *O. gracilis.* The embryo sac is of the Normal type and well developed endosperm haustoria are formed. Glišić emphasizes the affinity between the Scrophulariaceae and the Orobanchaceae. In his opinion, the resemblance in endosperm formation and seed development in these two families is so close that they could be united into one. Cassera (1935) described the development of the female gametophyte, endosperm, and embryo of *O. uniflora* and, like Glišić, concluded that the Orobanchaceae and Scrophulariaceae are closely related. Srivastava (1939) reported that in *O. aegyptiaca* the mature embryo sac is of the Normal type and the endosperm has an aggressive micropylar and a less active chalazal haustorium. Juliano (1935) studied the anatomy and morphology of *Aeginetia indica.* He found that the embryo sac is of the Normal type and the endosperm haustoria are vestigial.

[1]Quoted in Glišić, 1929 and Schnarf (1929).

MATERIALS AND METHODS

Cistanche tubulosa, the object of the present study, is a large total parasite attached to the roots of *Calotropis* and *Acacia*, as found in sandy places near Ajmer. This is probably the only species of *Cistanche* known from India (Hooker, 1875; Sabnis, 1941) and is found in Rajputana, Panjab and Sind (Fig. 1). It extends outside India to Central Asia and Arabia. At Ajmer the plant makes its appearance above ground in January and completes its life cycle in four to five weeks after which it sheds its seeds and dries up. Several shoots of the parasite originate from the same point. They run horizontally in the soil as rhizomes for a short distance, then become erect and rather

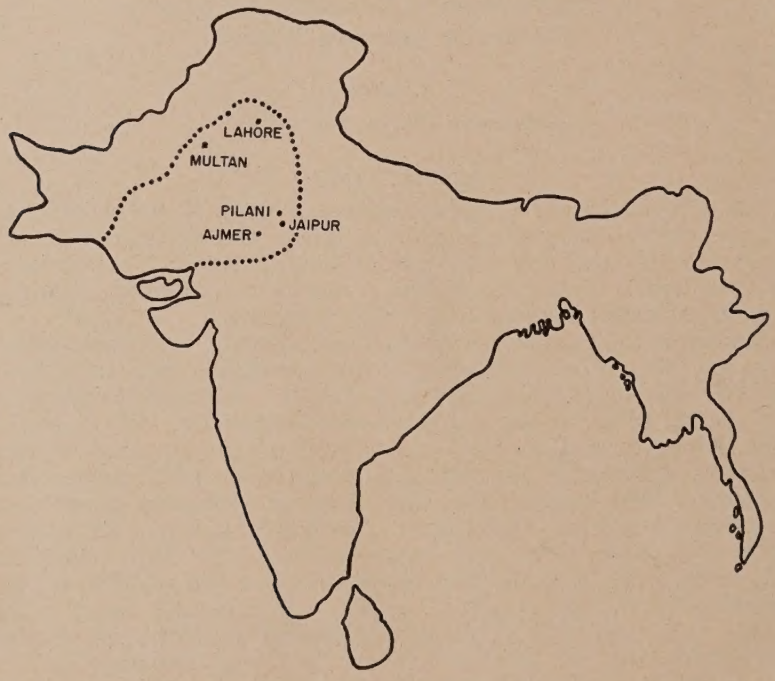

FIG. 1. Map showing distribution of *Cistanche tubulosa* in India and Pakistan.

suddenly produce scapigerous, scaly, unbranched and dense flowering spikes (Figs. 2–5). The scapes are yellowish tinged with purple and usually more than three feet in length. The rhizomes are about three inches in diameter, very turgid and densely packed with large starch grains.

EXPLANATION OF FIGURES 2–5

FIG. 2. Photograph showing underground parts of host (*Calotropis*) and parasite. FIG. 3. Plants of *Cistanche* attached to host (*Calotropis*). FIG. 4. Two detached plants of *Cistanche*. FIG. 5. Inflorescence axis of *Cistanche* more highly magnified.


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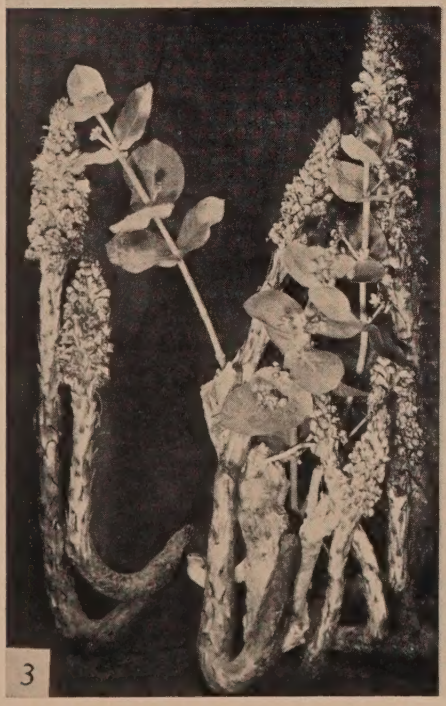
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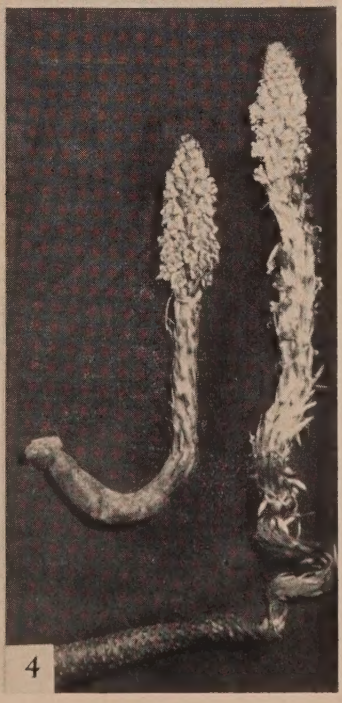
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The material was fixed in formalin-acetic-alcohol, Nawaschin's fluid and medium chrom-acetic acid, the first of which gave the best results. The older ovaries were cut into pieces in order to obtain good fixation.

Dehydration and infiltration were carried out as usual. Sections were cut 8–15 μ thick and stained either with safranin and fast green or with Haidenhain's iron-alum haematoxylin. Differentiation was carried out in the latter case with an aqueous solution of picric acid (Maheshwari, 1933). The sections take a long time to destain even when the slides are kept in haematoxylin for only a few minutes. Safranin and fast green proved very satisfactory and gave a very clear differentiation. For fastening the sections to the slide a mixture of 'gloy' and potassium dichromate in water was found to be very satisfactory (Maheshwari, 1930).

ORGANOGENY

The floral parts arise in acropetal succession: sepals, petals, stamens and carpels (*cf.* Juliano, 1935; Srivastava, 1939). The carpels appear as two outgrowths which fuse along their margins and form the

FIG. 6. Flower bud. x0.7. FIG. 7. Open flower, lateral view. x0.7. FIG. 8. Open flower, front view. x0.7. FIG. 9. Open flower, dissected to show stamens and gynoecium. x0.7.

ovary, style and stigma. The inner surfaces of the two carpels differentiate into two placentae (Fig. 10) each of which bifurcates into two (Figs. 11 and 12). The floral buds situated at the base of the scape are apparently the oldest but are buried in sand and, even if they later appear above ground level, they do not resume their normal development, as their floral parts are already collapsed and shrivelled.

THE FLOWER

The flowers are sessile, each being situated in the axil of a triangular bract which is longer than the calyx. There are two membranous lanceolate bracteoles, one on each side of the flower. The five sepals

are approximately one third as long as the corolla. The bilabiate corolla tube is wider above and bent forward. It consists of five yellowish purple lobes which are rounded at the apex and strongly reflexed. The stamens are didynamous (the two anterior being the larger), sub-exerted and epipetalous, the posterior stamen being absent (Figs. 6–9). They appear as small stumpy protuberances which soon differentiate into a short epipetalous filament and a massive anther. The outer surface of the anthers is woolly and they are closely adherent to each other resulting in a pseudo-syngenesious condition (Figs. 13, 14).[2] The ovary is bicarpellary and superior, with a very long style which is strongly curved below the bilobed, broad and glandular stigma. There are four T-shaped parietal placentae (Figs. 10–12), each bearing numerous anatropous ovules. Below the ovary there is a very conspicuous glandular disc. The fruit is a beaked and ovoid capsule about one inch in length. Dehiscence is by two valves along the antero-posterior margins of the carpels.

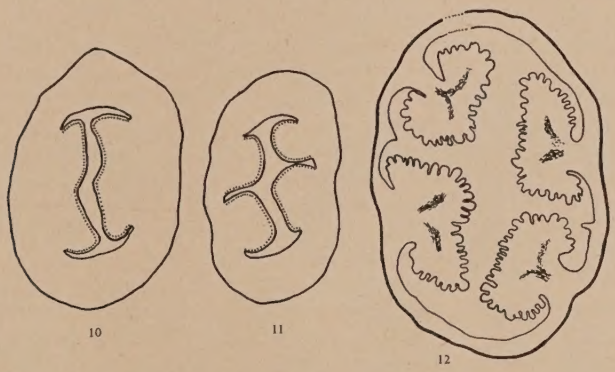

FIG. 10. T. S. young ovary showing the two parietal placentae. x423. FIG. 11. T. S. ovary showing bifurcation of the two placentae to form four. x423. FIG. 12. T. S. older ovary showing four parietal placentae with numerous ovular primordia. x15.

MICROSPOROGENESIS

The young anther consists of homogeneous cells and is circular in cross section but gradually it becomes four-lobed with a fifth rounded projection formed by the connective. The archesporium is hypodermal (Fig. 15) and forms four crescent-shaped structures with a massive columella-like structure forming the central portion of the anther (Fig. 13). In *Aeginetia* (Juliano, 1935), too, the archesporium is crescent-shaped but the anther is bilocular.

The outermost cells of the archesporium cut off the primary parietal layer (Fig. 15) which further divides to form the endothecium, two middle layers and tapetum (Figs. 16, 17 and 18). At some points more than two middle layers may be seen. When the microspore mother

[2]In *Aeginetia indica* (Juliano, 1935) the adhesion is attributed to a thick mucilaginous secretion produced by the glandular trichomes formed on the filaments near their attachment to the connectives.

cells are in synizesis, the inner middle layer becomes flattened and crushed (Fig. 18). The walls of the epidermal cells become slightly lignified. The endothecium and even the outer middle layer show much thickening and lignification (Fig. 19), but the fibrous thickenings which are characteristic of the former are absent.

The innermost parietal layer which is in contact with the sporogenous tissue functions as the tapetum. On the side towards the connective it seems to be derived from sporogenous cells. The tapetal cells are radially elongated and have dense cytoplasm and prominent nuclei. During the prophase of the first meiotic division the nuclei undergo one division and the cells thus become binucleate (Fig. 18). Each nucleus has two or more nucleoli. At certain places the tapetum becomes two-layered. During microspore formation the protoplasm of the tapetal cells becomes vacuolate, the walls become indistinct, and the cells seem to merge into each other. Only traces of the tapetum are seen at the microspore stage and it completely disappears afterwards. At maturity the wall of the anther consists of three layers only: the epidermis, endothecium, and outer middle layer. The two loculi on each side become continuous and the anther becomes bilocular. In *Aeginetia* (Juliano, 1935) only the endothecium and the epidermis persist. The endothecial cells become thick-walled but here too fibrous thickenings are absent.

The sporogenous cells divide to produce a U-shaped plate of microspore mother cells in each lobe of the anther (Fig. 13). In the resting condition the nucleus shows a fine reticulum with a large nucleolus. As it enters into the meiotic prophase, it increases in size, and a loose tangle of chromatin threads appears in which the nucleolus is still quite distinct. Next, the mother cells enter into the synizesis stage, in which the chromatin material shrinks to form a knot lying on one side of the nuclear membrane. The rest of the nucleus seems to be empty, although rarely it may be traversed by a few chromatin threads (Fig. 18). The bivalent chromosomes soon arrange themselves on the equator followed by the formation of the spindle (Fig. 20). While I am unable to give a definite count, the number of chromosomes is between 19 and 21. No wall is formed after the division (Fig. 21) but after a short

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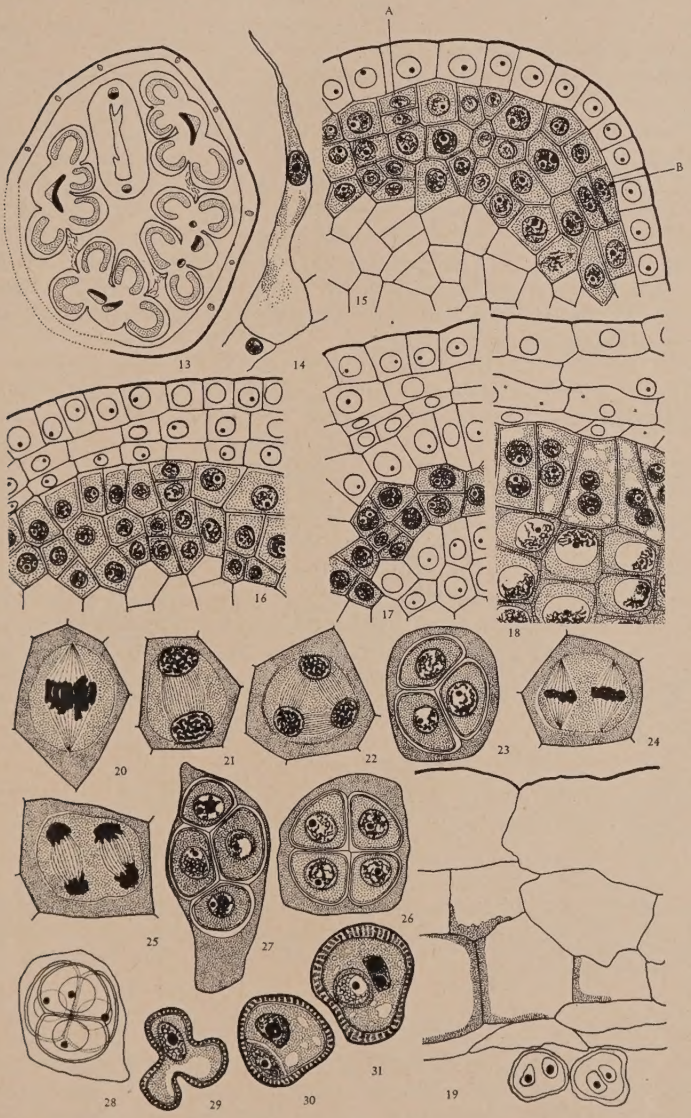
EXPLANATION OF FIGURES 13–31

FIG. 13. T. S. young flower bud; note interlocking of adjacent anthers resulting in a pseudosyngenesious condition. x29. FIG. 14. Single hair from outer surface of anther. x900. FIG. 15. T. S. part of anther lobe showing hypodermal group of archesporial cells. Note periclinal divisions in the outer layer at places marked *A* and *B*. x900. FIG. 16. T. S. anther lobe showing sporogenous cells and two parietal layers. x900. FIG. 17. Older stage showing sporogenous tissue and three wall layers. x900. FIG. 18. Still older stage showing the microspore mother cells in synizesis. The tapetum has become binucleate. x900. FIG. 19. Wall of mature anther. Note epidermis and thickened endothecium and outer middle layer. x900. FIG. 20. Microspore mother cell in metaphase. x1366. FIG. 21. End of Meiosis I. x1366. FIG. 22. End of Meiosis II. x1366. FIG. 23. Tetrad of microspores, note mucilaginous layer between cytoplasm and mother cell wall. x1366. FIGS. 24, 25. Second meiotic division with spindles arranged parallel to each other. x1366. FIG. 26. Isobilateral tetrad. x1366. FIGS. 27, 28. Decussate tetrads. x1366. FIG. 29. Pollen grain showing three furrows. x1366. FIG. 30. Two-celled pollen grain. x1366. FIG. 31. Same, older stage. x1366.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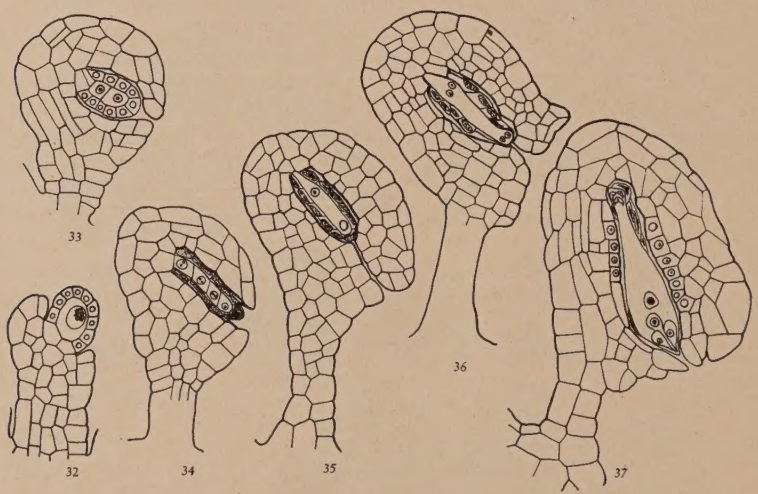
interval of interkinesis the two nuclei enter into the second meiotic division. The spindles may be parallel so as to produce an isobilateral tetrad (Figs. 24–26), or at right angles to each other resulting in a tetrahedral tetrad (Figs. 22, 23). Sometimes decussate tetrads are formed (Figs. 27, 28). Secondary spindles connect the four nuclei of the tetrad with one another.

Cytokinesis takes place by furrowing. The original wall of the microspore mother cells remains intact throughout the whole process of meiosis. A special wall, mucilaginous in nature, is secreted by the protoplast, inside the original wall. From it four equidistant peripheral furrows grow inwards, ultimately meeting in the center and dividing the protoplast simultaneously into four spores. The furrows are so narrow that they look almost like cell plates. A similar type of division has been described in *Lathraea* (Gates, 1925) and *Aeginetia* (Juliano, 1935). The microspores are liberated by the disintegration of the wall of the spore mother cell and the dissolution of the mucilaginous coat which surrounds the young tetrad.



FIGS. 32–37. Stages in inversion of the ovule. x440. FIG. 32. Megaspore mother cell stage. FIG. 33. Dyad stage. FIG. 34. Tetrad stage. FIG. 35. Two-nucleate embryo sac stage. FIG. 36. Four-nucleate embryo sac stage. FIG. 37. Mature embryo sac stage.

MALE GAMETOPHYTE

The microspore is filled with dense protoplasm and has a large centrally placed nucleus. The pollen grains are of the tricolpate type (Wodehouse, 1935) possessing three meridionally placed furrows (Fig. 29). This is the result of the tetrahedral arrangement in which each microspore makes contacts with the three adjoining cells at three points. As the cell increases in volume, its protoplasm becomes vacuolate and its nucleus is displaced towards the periphery, where it divides to produce the generative and tube cells (Fig. 30). The former soon

moves in and takes up a more central position (Fig. 31). It stains more deeply with haematoxylin than the vegetative cell.

OVULE

The ovules develop on the placenta as blunt, swollen and crowded protuberances (Fig. 12). As they grow they become anatropous by the quicker growth of the cells on one side (Figs. 32–37). They are tenuinucellate and unitegmic. The single integument is about six cells thick.

The nuclei of the nucellar epidermis are large enough to occupy almost the whole width of the cell. The chromatic material appears in lumps and there are one to two prominent nucleoli in each nucleus. During megasporogenesis the nucellar epidermis begins to disintegrate and all traces of it disappear during the growth of the embryo sac. Juliano states that "the nucellus of the megaspore undergoes enlargement soon after fertilization" and that "the enlarging endosperm then presses the nucellar cells against the massive testa and this nucellus disappears in the mature seed." This is evidently incorrect for he seems to have mistaken the inner layer of the integument for the nucellar epidermis.

As the nucellus disintegrates, the integumentary tapetum comes in contact with the embryo sac. In post-fertilization stages, it encloses the greater part of the embryo sac except its base and apex. The cells are large, closely packed and densely cytoplasmic with a very prominent nucleus. Cassera (1935) and Srivastava (1939) report that in *Orobanche uniflora* and *O. aegyptiaca* the cells often become binucleate. Their glandular structure seems to indicate that they are connected with the nutrition of the embryo sac and the endosperm. In later stages the cells become vacuolate, flattened and heavily cutinized, and serve a protective function.

MEGASPOROGENESIS

There is a single hypodermal archesporial cell, which functions directly as the megaspore mother cell (Figs. 38, 39). As it enlarges any nucellar cell or cells lying parallel to it disintegrate and disappear. The megaspore mother cell elongates and at the conclusion of the first meiotic division (Fig. 40) two dyad cells are produced (Fig. 41), both of which divide simultaneously (Fig. 42) to give rise to a linear tetrad of megaspores (Fig. 43). The spindles of this division are, however, so arranged as to produce a slightly curved tetrad. Occasionally T-shaped tetrads are also produced (Figs. 44, 45). A linear tetrad of megaspores has been reported in all other Orobanchaceae so far investigated, viz., *Christisonia* (Worsdell, 1897), *Orobanche minor* (Carter, 1928), *O. hederae* and *O. gracilis* (Glišić, 1929), *O. uniflora* (Cassera, 1935), *Aeginetia indica* (Juliano, 1935) and *Orobanche aegyptiaca* (Srivastava, 1939).[3]

[3]According to Bernard (quoted in Glišić, 1929) the megaspore mother cell in *Orobanche* frequently divides to produce four free nuclei without any walls between them. Glišić considers this to be improbable and I am in agreement with this opinion.

EMBRYO SAC

The chalazal megaspore functions and gives rise to the embryo sac while the three micropylar megaspores disintegrate and become absorbed after some time (Fig. 46). In *Orobanche minor* (Carter, 1928) the third

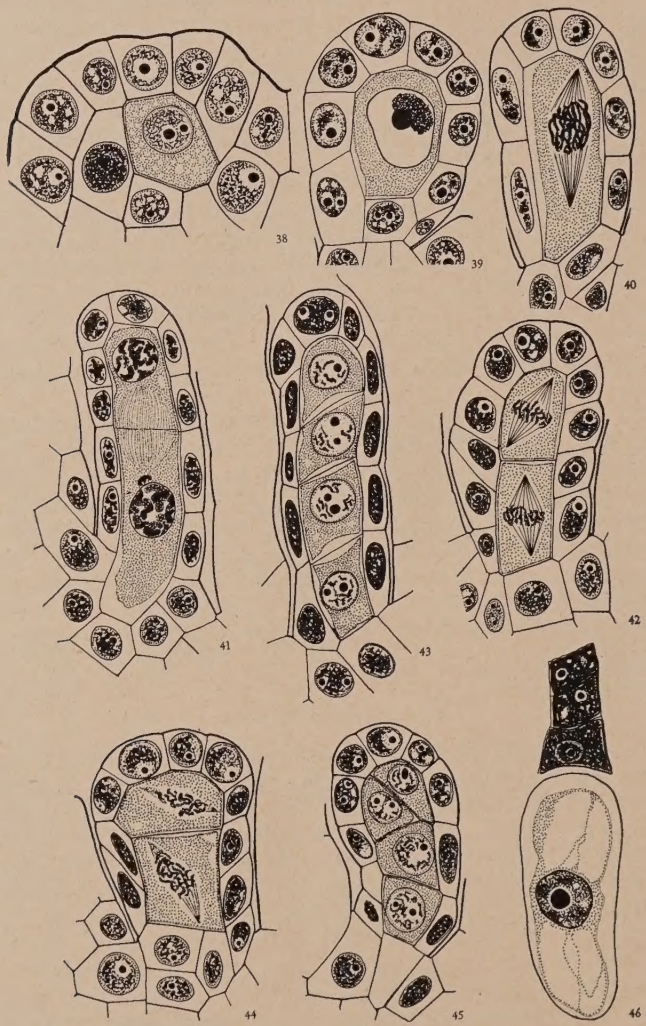
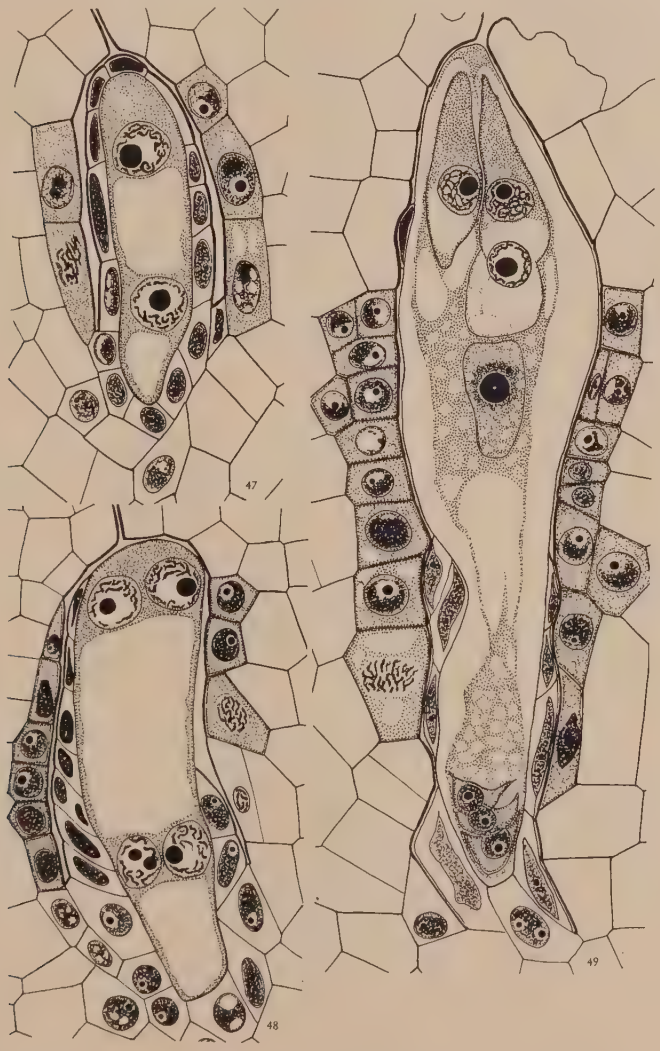


FIG. 38. L. S. nucellus showing primary archesporial cell. x1467. FIG. 39. Megaspore mother cell. x1467. FIG. 40. Megaspore mother cell dividing. x1467. FIG. 41. Late telophase of Meiosis I. x1467. FIG. 42. Second meiotic division. x1467. FIG. 43. Linear tetrad of megaspores. x1467. FIG. 44. Division spindles of Meiosis II oriented more or less at right angles to each other. x1467. FIG. 45. T-shaped tetrad. x1467. FIG. 46. Functioning megaspore with the three degenerated micropylar megaspores. x1467.

and the fourth, or the second and the fourth megaspores, may develop simultaneously. Srivastava (1939) mentions that the degeneration of the three micropylar megaspores does not follow a definite sequence. Usually it proceeds from the micropylar end downward but sometimes the second megaspore degenerates only after the first and the third. The nucleus of the functioning megaspore is located in the center and there is one large vacuole on either side of it in the long axis of the cell. The two daughter nuclei formed after the first division lie side by side



FIGS. 47, 48. Two-nucleate and four-nucleate embryo sacs. Note disintegration of nucellar epidermis and formation of integumentary tapetum. x1630. FIG. 46. Mature embryo sac. x1630.

FIG. 50. First division of primary endosperm nucleus resulting in the formation of the primary micropylar and primary chalazal chamber. x1200. FIG. 51. Vertical division of primary micropylar chamber. Chalazal chamber undivided. x1200. FIG. 52. The two micropylar cells dividing transversely. Chalazal chamber uni-nucleate. x1200. FIG. 53. Micropylar chamber showing two micropylar haustorial cells, a narrow isthmus region, central cells of endosperm proper and binucleate chalazal haustorium. The terminal cell of the three-celled proembryo is surrounded by the cells of the endosperm. x393. FIG. 54. Two-nucleate chalazal haustorium with antipodal cells still persisting at the base. x1200. FIG. 55. The chalazal haustorium crushed and reduced to a narrow streak. x1200.

for some time but ultimately migrate to their respective poles and a large vacuole is formed in the center. The two-nucleate stage (Fig. 47) is followed by the four- (Fig. 48) and eight-nucleate stages. The mature embryo sac is monosporic and eight-nucleate, designated as the "Polygonum type" by Maheshwari (1948). The chalazal end of the embryo sac is tapering and slightly curved so that the antipodals are rarely seen in the same section which shows the egg apparatus and the polar nuclei. The synergids are pyriform in shape with a prominent hook, filiform apparatus and large basal vacuole.[4] They begin to show signs of degeneration at the time the primary endosperm nucleus is dividing and only their disintegrated remains are seen during the early endosperm stages. The egg shows a large vacuole in its upper part and a smaller one in its lower. The two polar nuclei fuse near the egg apparatus to give rise to a large ovoid secondary nucleus. It has a prominent nucleolus which shows a distinct droplet-like structure in its center (Fig. 49). The antipodal cells are crescent-shaped and either arranged in a single row (Figs. 49, 51), or two of them lie side by side and the third lies above (Fig. 50) or below them (Figs. 52, 53, 54). They are recognizable for a long time after fertilization.[5] Their nuclei, however, are poor in chromatin and take a bluish stain with safranin and fast green. In many ovules of *Orobanche hederae* Glišić (1929) observed a protrusion of the broad micropylar portion of the embryo sac into the cavity of the ovary. This was, however, true only of such embryo sacs which remained unfertilized and whose cytoplasm and nuclei were in a state of degeneration. In *Cistanche* I never observed any appreciable protrusion of the embryo sac into the ovary cavity. There is only a slight protrusion into the micropyle which takes place after the disintegration of the nucellus.

POLLINATION AND FERTILIZATION

The flowers are protandrous. Stamens and stigma are subexerted and eventually lie at approximately the same level. Therefore self-pollination is not unlikely. The disc below the ovary secretes a sugary substance which attracts flies. Probably they serve to bring about cross-pollination (Figs. 7, 8, 9).

At the time of fertilization the synergids are about to disintegrate. Usually the pollen tube destroys one of them but sometimes both remain intact. The pollen tube contains a number of densely staining X-bodies. Both syngamy and triple fusion seem to take place at the same time.

ENDOSPERM

The endosperm is of the cellular type as in other Orobanchaceae. After fertilization the embryo sac enlarges. At this stage it shows the fertilized egg, the degenerating synergids, a large primary endosperm

[4]Juliano (1935) states that in *Aeginetia* as well as in other parasitic angiosperms the filiform apparatus is absent. However, since it is present in *Cistanche* and also in *Santalum* and *Viscum*, it is probable that there is no correlation between the occurrence of the filiform apparatus and the parasitic habit.

[5]This is also the case in other members of the family except *Orobanche minor* in which Carter (1928) reports that the antipodals degenerate long before the time of fertilization.

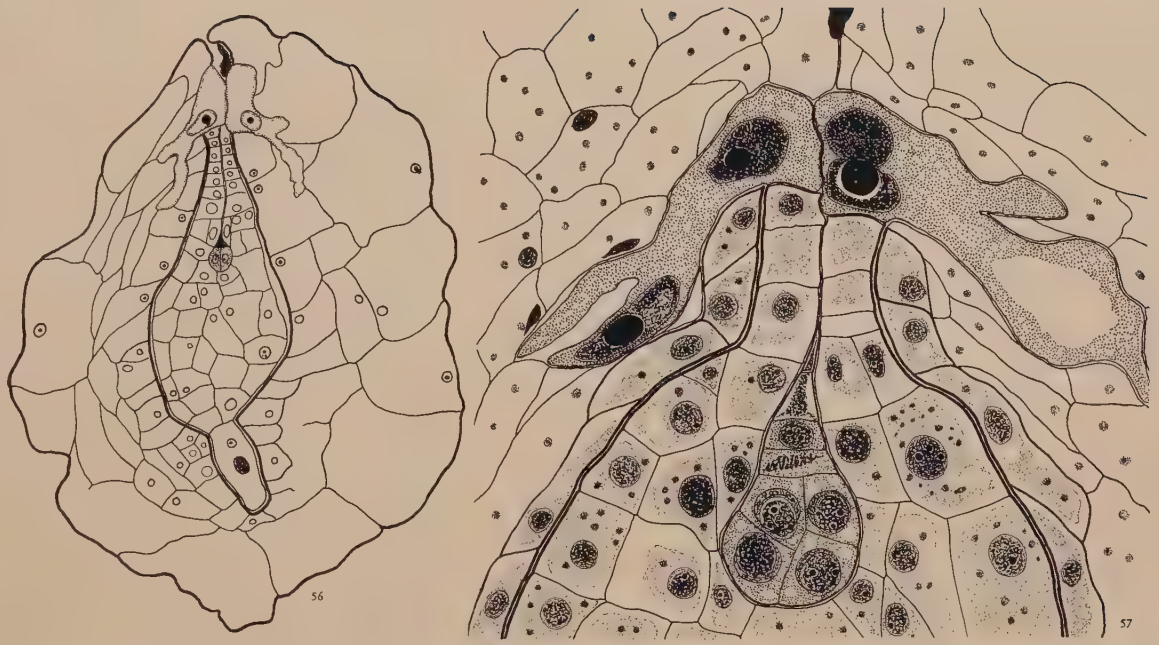


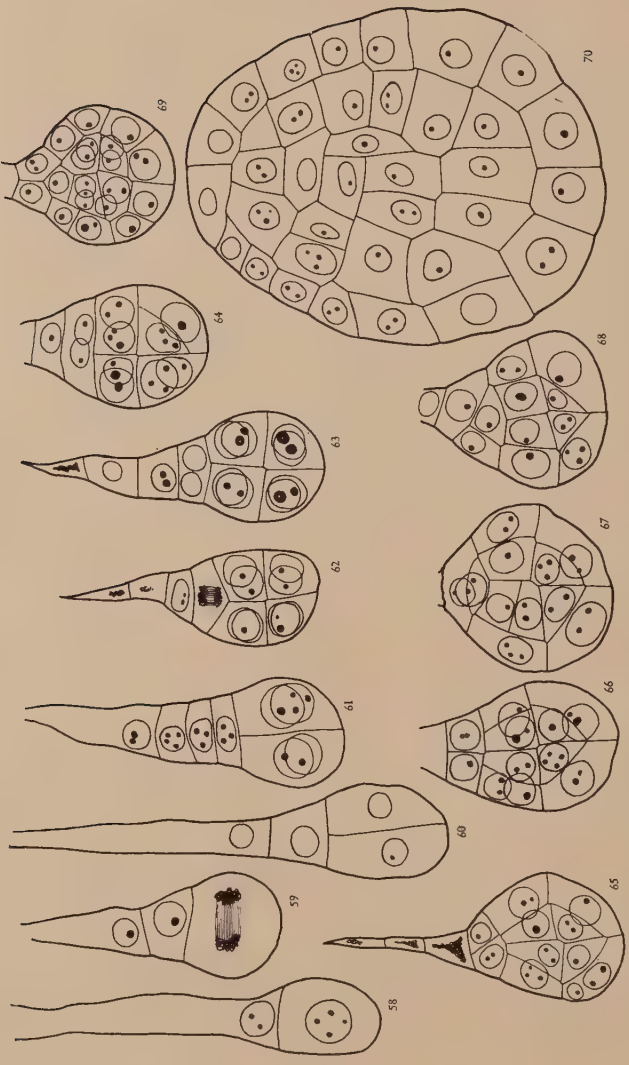
FIG. 56. L. S. young seed, showing integument, two celled micropylar haustorium, young embryo, and two-nucleate chalazal haustorium. x393. FIG. 57. Enlarged view of upper part of seed of slightly older stage showing micropylar haustorial cells with hypertrophied nuclei. Note starch grains in cells of integument and endosperm. x1200.

nucleus, and the three antipodal cells. The first division of the primary endosperm nucleus is followed by a transverse wall resulting in the formation of a micropylar and a chalazal chamber (Fig. 50). Cassera (1935) and Srivastava (1939) state that in *Orobanche* the first division of the micropylar chamber is longitudinal but sometimes it may be transverse and is then followed by a longitudinal division. Glišić (1929) believes that the first wall in the micropylar chamber is always longitudinal but is often overlooked when it lies in the plane of the section. This is also the case in *Cistanche* and a transverse division, if at all found, must be considered as an abnormality. The primary chalazal chamber does not divide further and directly functions as a weak chalazal haustorium. Its nucleus becomes hypertrophied and may show six or more nucleoli (Figs. 51, 52). It usually undergoes one division (Figs. 53, 54) but may sometimes remain undivided. In *Orobanche cumana* and *O. ramosa* (Persidsky 1926) three to four nuclei may be seen in the chalazal haustorium. In later stages it becomes pressed and squeezed by the enlarging cells of the integumentary tapetum and is flattened to a narrow canal (Fig. 55). The two daughter cells of the micropylar chamber divide transversely (Fig. 52) to cut off a micropylar haustorium consisting of two vesicular cells each of which contains a prominent hypertrophied nucleus. The cells below them divide to form a narrow isthmus region[6] consisting of two rows of approximately six cells each and a broader basal part which may be designated as the endosperm proper (Fig. 53). The densely staining cytoplasm and prominent nuclei of the isthmus cells suggest that they serve to conduct nutritive substances from the micropylar haustoria towards the endosperm proper. Glišić (1929) says that in consequence of their function as conductors of nutritive substances these cells are strongly overfed resulting in their disturbed growth and final degeneration. The micropylar haustorial cells are very active and aggressive and give out branched hypha-like intercellular processes which invade the tissues of the integument (Figs. 53, 56 and 57). They have dense cytoplasm and usually contain two nuclei one of which may migrate into the process. In some ovules Glišić observed an extra-micropylar growth of the micropylar haustorial cells whose branches penetrated the integument and passed out of it. In *Cistanche* they are quite prominent but I have not observed them to pass out of the integument, nor did I see any extra micropylar outgrowths. Juliano (1935) mentions that in *Aeginetia indica* the micropylar haustorial cells do not possess the dense cytoplasm and hypertrophied amoeboid nuclei which form such a constant feature of most of the Orobanchaceae. Here they seem to be degenerate structures. In later stages the micropylar haustorium and the isthmus region disappear and in their places only a narrow canal is seen, similar to the one at the chalazal end after the chalazal haustorium has been crushed by the integumentary cells.

EMBRYO

The oospore elongates and divides transversely into two cells, the micropylar of which forms a long tapering suspensor situated between

[6]Also called the "sterile" micropylar part of the endosperm.



FIGS. 58 to 70. Stages in the development of embryo. For explanation see text. x573.

the cells of the narrow isthmus region of the endosperm (Figs. 53, 58). The next stage shows a row of three cells, the terminal and the subterminal of which take part in the construction of the embryo (Fig. 59). Persidsky (1926) and Srivastava (1939) state that in the species of *Orobanche* studied by them the suspensor consists of a number of cells.

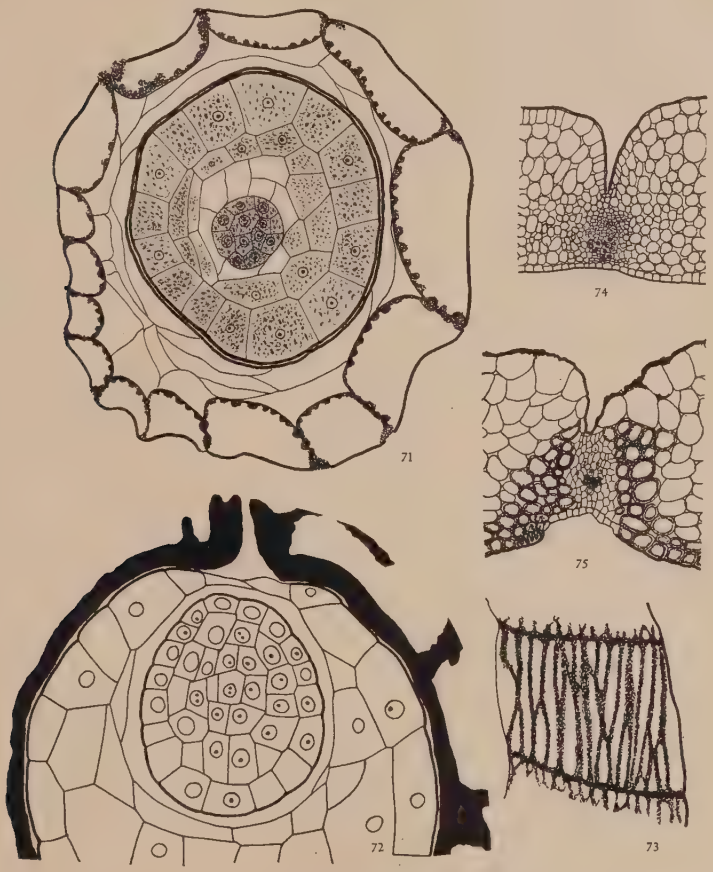


FIG. 71. T. S. seed showing endosperm, embryo and testa. x185. FIG. 72. L. S. upper part of the ripe seed, showing endosperm, embryo, and innermost wall of the testa. x474. FIG. 73. L. S. cell of testa showing spiral thickenings. x200. FIG. 74. T. S. part of ovary wall showing one of the grooves in the pericarp which marks the future line of dehiscence of the capsule. x200. FIG. 75. Older stage showing thickening in the cells on either side of the groove. x200.

Cassera (1935), on the other hand, writes that in *O. uniflora* it is always unicellular and is soon absorbed *by the embryo* (italics mine). I believe, however, that the cells of the suspensor disintegrate because of the pressure of the adjoining cells of the isthmus region of the endosperm.

The terminal cell of the proembryo divides by a vertical wall (Fig. 60). Further divisions result in the quadrant and octant stages (Figs. 61, 62, 63). Periclinal divisions now cut off the dermatogen, first in the anterior half (Fig. 64) and then in the posterior (Crété 1942). The lower cell of the suspensor also divides longitudinally (Fig. 66), transversely (Fig. 67), or obliquely (Fig. 65) to produce two cells. One of these daughter cells cuts off another cell which joins with the periblem and plerome while the two remaining cells complete the dermatogen at the radicular end of the embryo (Figs. 68, 69). The cells within the dermatogen undergo periclinal divisions to differentiate into the periblem and plerome, which in turn also grow by anticlinal divisions. The mature embryo is an ovoid structure without any differentiation of the embryonal organs (Fig. 70).

THE SEED

The seed is a minute globose structure comprising the testa, endosperm and embryo (Fig. 71). The embryo lies embedded in the upper part of the endosperm which stores large quantities of oil and starch. The remnants of the micropylar and chalazal haustoria are represented at this stage by two canals, one at the micropylar end in the isthmus region and the other at the chalazal end. The canal at the micropylar end is the beak-shaped micropyle (Fig. 72) and it is probable that during germination the embryo comes out through it. The inner wall of the integumentary tapetum becomes heavily cutinized and surrounds the endosperm as a protective envelope. The middle layers of the integument degenerate and disappear while the outermost layer becomes greatly enlarged and develops lignified thickenings which are laid down in spirals but later becomes reticulate and even pitted (Fig. 73). Ovules are frequently checked in their development at almost any stage after the differentiation of the mother cells and several empty seeds are produced without embryo or endosperm. However, even in such ovules the integument continues its normal development and gives rise to a testa. The fruit capsule dehisces antero-posteriorly along two furrows seen on the pericarp (Fig. 74). At the time of dehiscence the cells of the pericarp situated on either side of the groove become spirally thickened (Fig. 75). The spirals may be so close as to produce a reticulate or even pitted appearance. These thickened cells cause a tension which results in the opening of the fruit.

CONCLUSION

Certain Orobanchaceae and Scrophulariaceae are quite similar in habitat, appearance, and flower structure. This is also true of the development of the male and female gametophytes, endosperm, and embryo. The formation of endosperm haustoria is common to both and the structure of the seed-coat is also very similar. All these characters speak for a close alliance between the two families, but since I hope to study other members of both, I wish to postpone a detailed discussion of their affinities to a later date.

SUMMARY

1. *Cistanche* is a large root parasite of the family Orobanchaceae, occurring on the roots of *Calotropis* and *Acacia*.

2. The floral parts develop in acropetal succession.

3. The anthers become pseudosyngenesious by the interlocking of the hairs formed by the proliferation of the epidermal cells of the anthers.

4. The anther is four-lobed. The archesporium is hypodermal.

5. The anther tapetum is of the glandular type and its cells become binucleate. At certain points it comprises more than one layer.

6. Quadripartition of the microspore mother cells takes place by furrowing. Tetrahedral, isobilateral, and decussate types of tetrads occur.

7. The pollen grain is two-celled. The exine is smooth and bears three furrows.

8. The ovules are tenuinucellate, anatropous and unitegmic. The nucellar epidermis degenerates and an integumentary tapetum is formed from the innermost layer of the integument.

9. The hypodermal archesporial cell functions directly as the megaspore mother cell without cutting off a parietal cell.

10. The embryo sac is of the "Polygonum" type. It is broader towards the micropylar end and tapers toward the chalazal. The antipodals are persistent but the synergids disappear soon after fertilization.

11. The endosperm is cellular. The first wall is transverse resulting in a primary micropylar and a primary chalazal chamber. The nucleus of the chalazal chamber divides but once resulting in a weak two-nucleate chalazal haustorium. The micropylar chamber divides longitudinally and then transversely so as to cut off a two-celled micropylar haustorium. The middle tier, by further transverse and longitudinal divisions, gives rise to a narrow isthmus region and the endosperm proper. The micropylar haustorium is very aggressive and sends intercellular hypha-like branches into the integument. The endosperm cells store starch and oil.

12. The proembryo consists of a three-celled filament. The suspensor cells soon disintegrate except the subterminal one which along with the terminal cell by further divisions gives rise to an ovoid embryo in which the dermatogen, periblem, and plerome are clearly marked out but there is no differentiation into the radicle, plumule, and cotyledons.

13. The seeds are minute and produced in large numbers. The cells of the testa are reticulately thickened. Many of the seeds have neither embryo nor endosperm but a well-developed seed coat is present.

ACKNOWLEDGMENT

I take great pleasure in expressing my sincere thanks to Prof. P. Maheshwari and Dr. B. M. Johri of Delhi University for the help rendered by them during the progress of this work. Thanks are also due to Dr. Bahadur Singh (Agra) and Mr. K. Subramanyam (Bangalore) for helpful suggestions. To Principal Kanhyalal Mathur of my college I am especially grateful for providing every facility for the work and for his constant encouragement and kindness.

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Contributions to the Embryology of Liliaceae. III. Embryogeny and Development of the Seed of Asphodelus tenuifolius Cav.

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INTRODUCTION

Although the development of male and female gametophytes has been studied extensively in various members of the Liliaceae, comparatively little is known of embryogeny in this family. Apparently the only detailed studies are on *Anthericum ramosum*, *Allium ursinum* and *Muscari comosum* (Souèges, 1931, 1932). The development of the embryo in these plants follows the Asterad type of Johansen (1945). *Erythronium dens-canis* (Guérin, 1931) follows the Onagrad type of Johansen (1945). As no detailed investigation has so far been carried out on the embryogeny of plants belonging to the sub-family Asphodeloideae, this study was undertaken to investigate the sequence of embryo development and seed formation in *Asphodelus tenuifolius*.

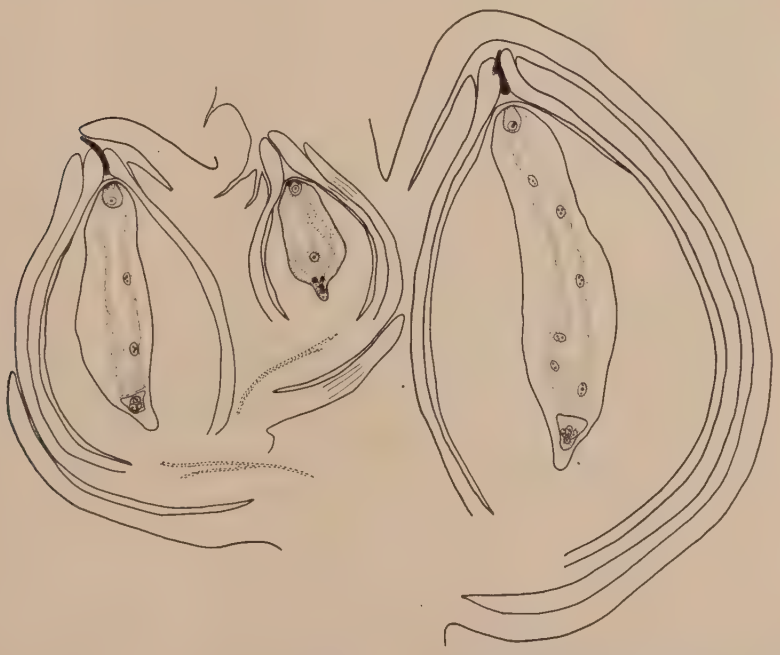


Fig. 1. L. S. ovule at the time of fertilization showing the aril. x123.
Figs. 2, 3. Further stages in the development of the aril. x123.

OVULE

The structure of the ovule of this plant has already been described by Stenar (1928) and by Maheshwari and Singh (1930). The chief peculiarity is the appearance of an aril from the basal portion of the ovule (Fig. 1). Its growth is greater on the outer surface of the ovule whereas on the inner surface adjacent to the funiculus it is comparatively slower (Fig. 2). At an older stage when a larger number of endosperm nuclei has been formed in both chambers, the ovule is completely covered by the aril (Fig. 3) which is made up of four layers of compactly

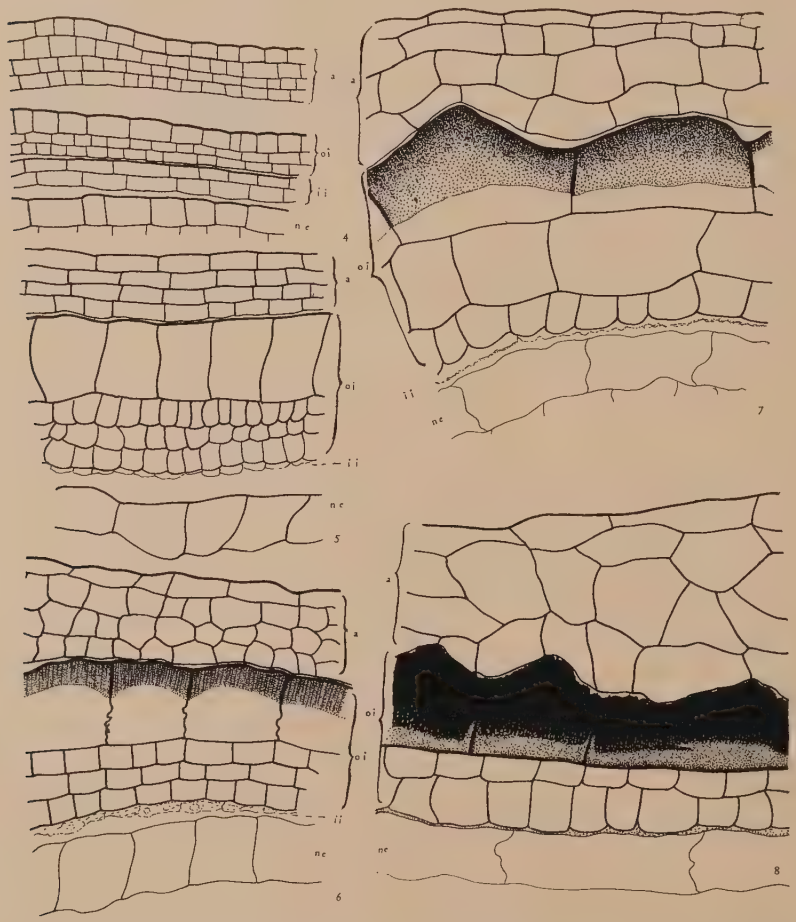


FIG. 4. A portion of the aril and the integuments enlarged. The nucellar epidermis is also shown (*a*—aril; *oi*—outer integument; *ii*—inner integument; *ne*—nucellar epidermis). x323. FIG. 5. Later stage showing the degeneration of the inner integument and enlargement of outer epidermal layer of outer integument. x323. FIGS. 6–8. Later stages of same showing gradual increase in deposition of thickening substance in the outer epidermal layer of outer integument. x323.

arranged thin-walled cells. Throughout its course the aril is quite free from the outer integument of the ovule. During later stages (Figs. 7–8) the cells of the inner layers of the aril gradually become considerably larger in size.

The presence of an aril has also been reported in *Bulbine*, *Eremurus* (Stenar, 1928), and *Asphodeline* (Schnarf and Wunderlich, 1939). In the first two genera the aril begins to develop at the two-nucleate stage of the embryo sac.

SEED COAT

At the time of fertilization (Fig. 4) the outer integument consists of three layers, the outermost of which is made up of larger cells. The inner integument is two-layered and consists of thin-walled cells of uniform size running close to the nucellar epidermis. At a slightly later stage (Fig. 5) the epidermal cells of the outer integument enlarge towards their outer surface and thus come to lie in close proximity with the innermost layer of the aril. Sometimes the cells of the inner layer of the outer integument divide periclinally so that the latter becomes four-layered. The inner integument gradually degenerates (Fig. 5). The cells of the outer epidermis of the outer integument become further enlarged and their external walls begin to thicken. The thickening is deposited first on the outer wall and then progresses inwards assuming a striated appearance (Fig. 6). In Fig. 7 the thickening has extended to more than half of the breadth of the cells and owing to increased deposition of the thickening matter the striations have now become less prominent. By the time the embryo is mature, the lumen of the cells becomes very narrow (Fig. 8).

The seed coat which is actually formed by the aril and the outer integument presents a rugose appearance (Fig. 9). In the mature seed the outer layer of the outer integument is deep black and, as the cells of the aril are colorless and transparent, the seeds appear shiny black.

ENDOSPERM

The endosperm is of the Helobial type (Maheshwari, 1933). The division of the primary endosperm nucleus which is situated in the chalazal region of the embryo sac is followed by the laying down of a transverse wall resulting in a large micropylar and a small chalazal chamber (Fig. 10). The next division is simultaneous in both chambers but further divisions take place more rapidly in the micropylar chamber. At the stage, when eight free nuclei are formed in the micropylar chamber, four are seen in the chalazal chamber (Fig. 3). The next division takes place only in the micropylar chamber resulting in 16 free nuclei whereas the chalazal chamber remains quadrinucleate (Fig. 12). One case was noticed in which the micropylar chamber had 16-nuclei but the chalazal had only two although these nuclei were larger than usual (Fig. 13). Further divisions in the micropylar chamber are rapid and all resulting nuclei become parietally disposed by the formation of a large central vacuole. Wall formation takes place at this stage (Fig. 14). Further growth of the endosperm is centripetal and results in the formation of a large mass of endosperm tissue (Fig. 9).

At the time of cytokinesis in the micropylar chamber, the chalazal chamber, which remains quadrinucleate, presents a rather conspicuous appearance (Fig. 15). The four nuclei become hypertrophied and are surrounded by dense cytoplasm. The basal portion of the chalazal chamber grows down in the form of a wedge-shaped structure disorganizing all tissues in the chalazal end of the ovule (Fig. 15). Its behavior in relation to the surrounding tissues indicates that it has a haustorial function. The chalazal chamber persists even in fairly old seeds (Fig. 9).

In *Asphodelus fistulosus* also, the chalazal chamber is four-nucleate; in *Bulbine annua* it is two-nucleate; and in *Eremurus himalaicus* it has 30–32 nuclei (Stenar, 1928).

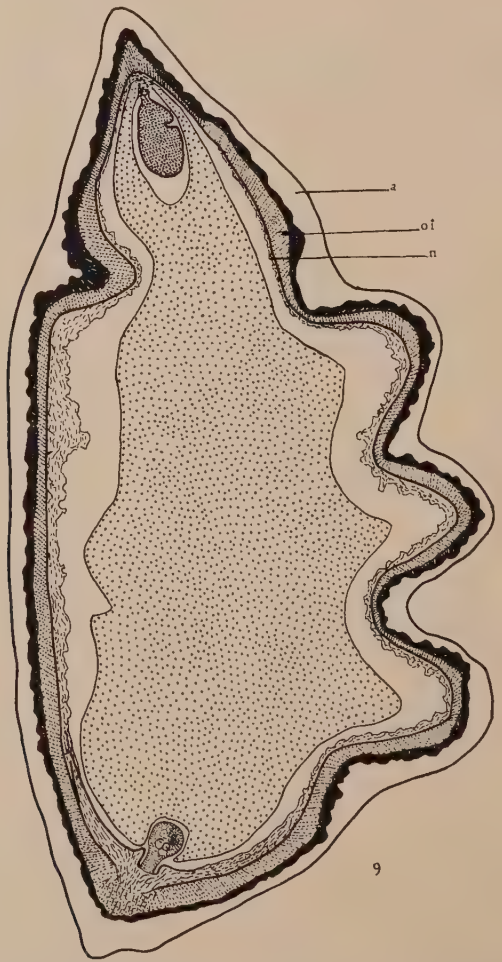


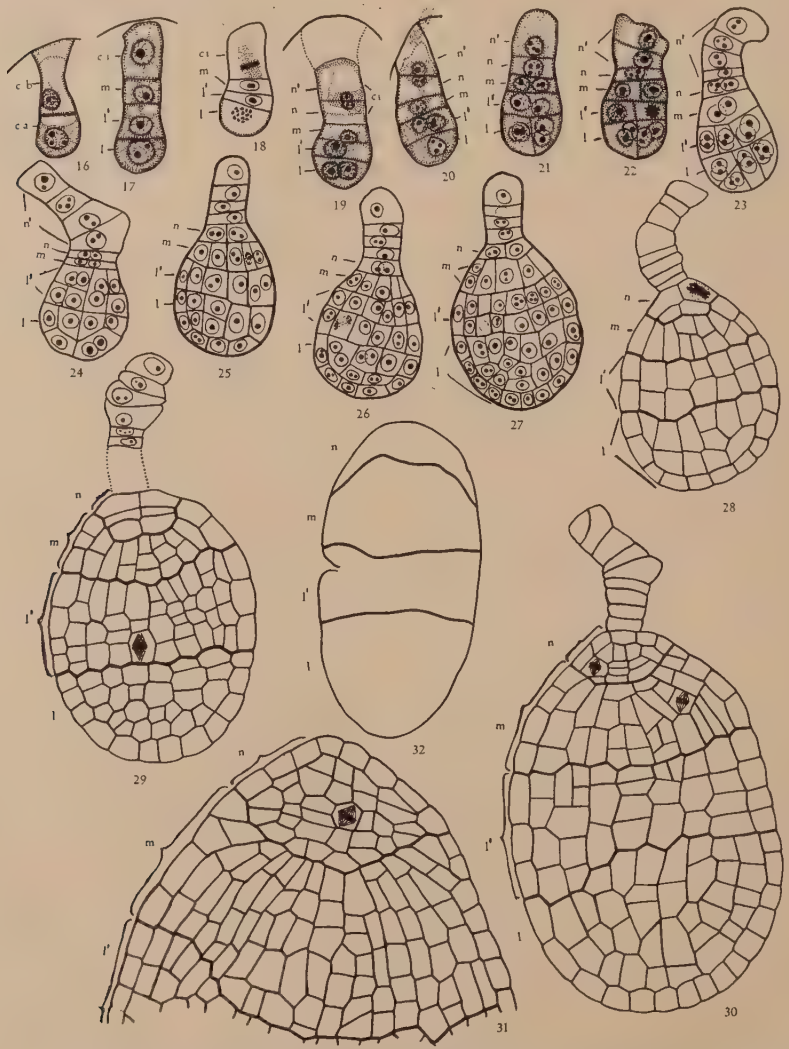
FIG. 9. L. S. seed showing seed coat, embryo, endosperm and chalazal chamber (*a*—aril; *oi*—outer integument; *n*—degenerated nucellus). x50.

EMBRYO

The fertilized egg elongates and divides transversely producing two cells *ca* and *cb* (Fig. 16). The next stage shows a filamentous proembryo of four cells *l*, *l'*, *m*, and *ci* (Fig. 17). The basal cell *ci* divides transversely forming *n* and *n'* and this is almost simultaneously followed by a vertical division in the terminal cell *l* (Figs. 18, 19). Cases were also noticed where division had taken place first in the subterminal cell *l'* (Fig. 20). Next the two cells in tier *l* undergo a vertical division at right angles to the first resulting in a group of four cells (Fig. 21).

FIGS. 10–14. Stages in development of the endosperm. Figs. 10–13, x172; Fig. 14, x40. FIG. 15. Enlarged view of lower part of seed, showing the chalazal endosperm chamber with hypertrophied nuclei. x388.

By this time the third cell *m* has also undergone a similar vertical division (Figs. 21, 22). A few more divisions occur in cells *l* and *l'*, delimiting the dermatogen (Figs. 23, 24), and the fourth cell *n* undergoes a vertical division (Figs. 23–25). Cell *n'* produces the suspensor (Figs. 22–30). Meanwhile, additional anticlinal and periclinal divisions occur in the first three tiers *l*, *l'* and *m* and the embryo now presents an ovoid appearance (Figs. 25–29). During further development tier *l* gives rise to the cotyledon, *l'* to the lateral stem tip, *m* to the hypocotyl, and *n* to the root tip.



FIGS. 16–22. Stages in development of the embryo (for explanation, see text). Figs. 16–27, x388; Figs. 28–31, x462, and Fig. 32, x206.

As seen in Figs. 28–29 the two cells of tier *n* divide periclinally, the inner set completing the periblem and the outer set the dermatogen. Soon periclinal walls are laid down in the outer two cells followed by similar divisions in the inner cells (Figs. 30, 31). The outermost cells form the root cap by further periclinal and anticlinal divisions (Fig. 31). The suspensor consisting of 8–9 cells persists almost up to this stage (Figs. 24–30), but soon begins to disintegrate.

The mature embryo with its terminal cotyledon and lateral stem tip is shown in Fig. 32. The centrally situated cells present an elongated appearance indicating a differentiation of the plerome.

SUMMARY

1. The endosperm is of the Helobial type. The chalazal chamber is tetranucleate. It functions as an haustorium in later stages and persists even during the development of the seed.

2. The aril envelops the developing seed completely.

3. The inner integument disorganizes during the early development of the seed. The cells of the outer epidermis of the outer integument enlarge and become filled with dark contents. This layer comes in intimate contact with the innermost layer of the aril.

4. The young proembryo comprises a filament of four cells, *l*, *l'*, *m* and *ci*; *ci* divides transversely to form *n* and *n'*. During further development tier *l* produces the cotyledon, *l'* the stem tip, *m* the hypocotyl, *n* the root tip and *n'* the suspensor.

ACKNOWLEDGEMENT

I am grateful to Prof. P. Maheshwari and to Mr. K. Subramanyam under whose guidance this work was done. I am also thankful to Dr. B. M. Johri and Mr. R. Khan for their kindness and encouragement. To the authorities of Delhi University I am grateful for the use of laboratory facilities during my stay at Delhi.

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The Genus Elaeagnus in Formosa

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Elaeagnus is the only genus of the family Elaeagnaceae in Formosa. In this study, eight species are recognized from Formosa and the adjacent small islands. Two of the species are proposed as new, and the synonymy of a few species is adjusted. This study is based on specimens deposited in the herbarium of the National Taiwan University, Formosa, and the U. S. National Herbarium, Smithsonian Institution, indicated by the abbreviations NTU and US respectively. Only selected specimens are cited from the former herbarium. The key is constructed on obvious vegetative characters only in order to expedite identifications in both the herbarium and the field.

KEY TO THE SPECIES

A. Leaves small, not more than 4 cm. long, distinctly obovate.
  B. Leaves rounded to slightly emarginate at apex.....1. *E. oldhamii*
  BB. Leaves acute at apex.....2. *E. obovata*
AA. Leaves more than 4 cm. long, lanceolate to oblong-ovate.
  B. Leaves chartaceous.
    C. Leaves acute to acuminate at apex; petioles short, 6–8 mm. long.
      D. Leaves silvery underneath.....3. *E. thunbergii*
      DD. Leaves brownish underneath.....4. *E. glabra*
    CC. Leaves rounded to obtuse at apex; petioles long, 1.5–2 cm. long.....5. *E. wilsonii*
  BB. Leaves thickly coriaceous, obtuse to acute or acuminate at apex.
    C. Leaves ovate to lanceolate, large, about 4–12 cm. long and 2–3.5 cm. broad, acute or acuminate at apex, the margins usually not revolute.
      D. Leaves broadly ovate, about 2–2.5 times as long as broad, acute at apex.....6. *E. macrophylla*
      DD. Leaves lanceolate to oblong-lanceolate, about 3 or 4 times as long as broad acuminate at apex.....7. *E. morrisonensis*
    CC. Leaves elliptic, smaller, 4–5 cm. long and 1.5–2.5 cm. broad, rounded to obtuse at apex, the margins usually revolute.....8. *E. formosana*

1. Elaeagnus oldhamii Maxim. in Bull. Acad. Sci. Pétersb. **15**: 377. 1871. Kanehira, Formos. Trees rev. ed. 487. *f. 448*. 1936.

Elaeagnus convexolepidota Hay. Icon. Pl. Formos. **9**: 88. 1920.

Endemic, very common in thickets at low altitudes throughout the island.

Formosa: Senkyakuseki coast, Sintiku-syu, *T. Hosokawa*, Dec. 27, 1941 (NTU); Kagi, *E. H. Wilson 9897* (US); Tetana, Sintiku, *E. H. Wilson 10308* (US); Musha, Nanto, *E. H. Wilson 11137* (US).

A small tree; branchlets lepidote; leaves thickly coriaceous, obovate, 3–4 cm. long, 1.3–2.3 cm. broad, rounded and often emarginate at apex, acute at base, the margins entire, green-lepidote above, silvery-lepidote

mixed with brown dots beneath, the costa indistinct above, raised beneath; veins and reticulations indistinct on both surfaces; petioles 3–5 mm. long, sulcate above, lepidote. Flowers 2 or 3, axillary; pedicels 4–6 mm. long including the globose ovary, silvery-lepidote. Calyx 4–5 mm. long, 4-lobed, silvery-lepidote without.

This species is distinctly characterized by the obovate leaves with rounded to slightly emarginate apices. The lower surfaces of the leaves are white-silvery-lepidote. A variety, var. *nakaii*, Hay. Icon. Pl. Formos. **2**: 127. 1912, was described from material collected in the Koishikawa Botanical Garden in Japan, said to have been brought from Formosa together with the typical form of the species, which was also in cultivation there. This variety has narrower, oblanceolate leaves, about 8–8.5 cm. long and 2–2.2 cm. broad.

Elaeagnus convexolepidota Hay. was given in the synonymy by both Sasaki (in Trans. Nat. Hist. Soc. Formos. **18**: 168. 1928) and Kanehira, who have had access to an isotype.

2. **Elaeagnus obovata** sp. nov.

Frutex, plus minusve spinescens; ramis cinereis, ramulis annotinis squamis argenteo-albidis obtectis; foliis membranaceo-chartaceis, obovatis, 3–4 cm. longis, 1.4–1.8 cm. latis, apice acutis, basi attenuatis, margine integris, supra initio argenteo-lepidotis, mox glabris, valde atris, enitidis, subtus dense et adpresse argenteo-lepidotis, squamis brunneis nullis, costa supra impressa subtus prominenter elevata, venis lateralibus utrinsecus circiter 5, supra vix conspicuis, subtus leviter elevatis, venularum rete inconspicuo; petiolis 3–4 mm. longis, supra canaliculatis, argenteo-lepidotis; floribus ignotis; fructibus 2–4-fasciculatis, axillaribus, ellipsoideis, 7–8 mm. longis, 3 mm. diametro, plus minusve costatis, dense argenteo-lepidotis; pedicellis 3–4 mm. longis, argenteo-lepidotis.

Formosa: Piyanan-ambu; Rato-gun, Taihoku-syu, *S. Hibino & Zyo-En Ko*, June 30, 1938 (NTU, type).

This species is distinctly characterized by the small obovate leaves, which are very dark above and silvery-white beneath, without the presence of any brown spots. The obovate leaves are readily distinguished from those of the common and widespread *E. oldhamii*, as they are thin, acute at apex, strongly discolored on the two surfaces, and without brown spots.

3. ELAEAGNUS THUNBERGII Serv. in Bull. Herb. Boiss. II. **8**: 384, 1908; Kanehira, Formos. Trees rev. ed. 487. *f. 449*. 1936.

Elaeagnus erosifolia Hay. Icon. Pl. Formos. **9**: 88. 1920.
Elaeagnus longidrupa Hay. op. cit. 90. f. 32, 1.
Elaeagnus oiwakensis Hay. op. cit. 92.

A scandent deciduous shrub. Leaves thickly chartaceous, ovate to elliptic, very variable in size, generally 4–5 cm. long and 2–2.5 cm. broad, sometimes to 8 cm. long and 3 cm. broad, acute at apex, acute to obtuse or rounded at base, entire at margins, green and glabrous above, silvery-lepidote with scattered brown spots beneath, the costa slender, subdistinct, the lateral veins 7 or 8 per side, the veins and reticulations indistinct; petioles 6–7 mm. long, lepidote. Flowers

2 or 3 or more in fascicles; pedicels 2–3 mm. including the ovary, brown- and silvery-lepidote; calyx brown- and silvery-lepidote, campanulate, 4–5 mm. long.

Endemic; widely distributed from low to medium altitudes.

Formosa: Hokotu, Sitisei-gun, *N. Murakami 232* (NTU); Sozan, Taihoku, *E. H. Wilson 11212* (US); Arisan, *E. H. Wilson 10881* (US); Happo, Sintiku, *E. H. Wilson 10322* (US); Arisan to Mt. Morrison, *E. H. Wilson 10039* (US), *R. Kanehira 3039* (US); Musha prov. Nanto, *E. H. Wilson 10037* (US), *15034* (US).

Elaeagnus thunbergii Serv. resembles *E. glabra* in the general shape and texture of the leaves, but it is readily distinguished from the latter species by the densely silvery-lepidote lower surfaces of the leaves. It also resembles *E. formosana* Nakai, but it has more acute and much thinner leaves. In *E. formosana*, the leaves are thickly coriaceous, with usually revolute margins and brownish-lepidote lower surfaces.

Elaeagnus erosifolia Hay. and *E. longidrupa* are given as synonyms by Kanehira, Sasaki (in Trans. Nat. Hist. Soc. Formos. *18*: 168. 1928), and Nemoto (Fl. Jap. Suppl. 508. 1936). *Elaeagnus oiwakensis* Hay. and *E. grandifolia* Hay. are listed in the synonymy by Sasaki and Nemoto, but these two names are not taken up by Kanehira. Sasaki also gives in the synonymy *E. morrisonensis* Hay., but this represents, as rightly noted by Kanehira, a distinct species; *E. grandifolia* is better referred to it than to *E. thunbergii*.

4. ELAEAGNUS GLABRA Thunb. Fl. Jap. 1784; Kanehira, Formos. Trees rev. ed. 484. *f. 445.* 1936.

Elaeagnus buisanensis Hay. Icon. Pl. Formos. **9**: 87. *f.* 31, *I.* 1920
Elaeagnus daibuensis Hay. op. cit. 88. *f.* 31, *V.*
Elaeagnus paucilepidota Hay. op. cit. 92. *f.* 32, *VI.*

A scandent shrub to 6 m. high. Leaves chartaceous, elliptic-ovate, 4–7 cm. long, 2.5–3.5 cm. broad, acutish to acuminate at apex, broadly cuneate at base, glabrous and dark-lustrous above, brownish-lepidote beneath, the costa impressed above, raised beneath, the veins and reticulations indistinct; petioles 6–8 mm. long, lepidote. Flowers 5-fasciculate, axillary; pedicels 3–4 mm. long; ovary ellipsoid, 6–7 mm. long; calyx about 7 mm. long, the tube about 4 mm. long, the lobes ovate, about 3 mm. long. Fruits ellipsoid, 1.2–2 cm. long, about 8 mm. across, gray or ferrugineous, the stalk about 4–10 mm. long.

From China to Liukiu and Japan.

Formosa: Rokutyori, Taihoku, *S. Suzuki 4188* (NTU); Lake Candidus, *E. H. Wilson 9987* (US).

The three synonyms are given by both Sasaki and Kanehira, who have had access to isotypes.

5. **Elaeagnus wilsonii** sp. nov.

Frutex scandens, 3–7 m. altus; ramis inermis, brunneis, ramulis annotinis brunneo-lepidotis; foliis membranaceo-chartaceis, late ovatis, 4–7.5 cm. longis, 2.5–5 cm. latis, apice et basi rotundatis, margine integris vel leviter undulatis, supra initio argenteo-lepidotis, mox glabris viridibus, enitidis, subtus squamis argenteis interdum fulvis intermixtis obtectis, costa supra inconspicua subtus leviter elevata,

venis indistinctis, venularum rete inconspicuo; petiolis 1–2 cm. longis, supra canaliculatis, brunneo-lepidotis; floribus ignotis; fructibus axillaribus, solitariis, ellipsoideis, circiter 1 cm. longis et 4–5 mm. diametro, aurantiaco-rubro-lepidotis, plus minusve costatis; pedicellis circiter 6 mm. longis, brunneo-lepidotis.

Formosa: Sozan, Taihoku-syu, common in thickets, *E. H. Wilson 10778*, Oct. 11, 1918 (US, type).

This species is close to *E. thunbergii* Serv., but is readily distinguished by its broader leaves, rounded at both ends, and its much longer petioles.

6. ELAEAGNUS MACROPHYLLA Thunb. Fl. Jap. 67, 1784; Kanehira, Formos. Trees rev. ed. 486. *f. 447.* 1936.

Elaeagnus kotoensis Hay. Icon. Pl. Formos. **9**: 90. 1919. *Syn. nov.*

A scandent shrub, to 3 m. high. Leaves coriaceous, broadly elliptic to broadly ovate, 5–12 cm. long, 4–8 cm. broad, obtuse to acute at apex, acute to rounded at base, green and scaly above, soon glabrous, densely appressed-silvery-lepidote beneath; the costa slightly raised on both surfaces, the lateral veins about 6 per side, slightly raised on both surfaces, the reticulations inconspicuous; petioles stout, 1–1.6 cm. long, lepidote. Flowers 4–6-fasciculate, axillary, pendulous; pedicels 3–6 mm. long including the globose ovary; calyx campanulate, about 7 mm. long and across, silvery-lepidote, the lobes 4, triangular, about 3 mm. long. Fruit ellipsoid, about 1.5 cm. long, brownish red when mature.

Japan and Korea to Liukiu Islands. In Formosa found only in Botel Tobago and Kashoto Islands.

Formosa: Kashoto, Churo, *Y. Kudo & K. Mori 272* (NTU).

This species is characterized by the relatively thickly coriaceous leaves, with silvery-lepidote scales on the lower surfaces and with acute apices and bases. The Formosan plant, originally referred to *E. kotoensis* Hay., has slightly narrower leaves than those from Japan but is not otherwise different.

7. ELAEAGNUS MORRISONENSIS Hay. in Journ. Coll. Sci. Tokyo **30** (1): 259. 1911 (Mat. Fl. Formos.); Kanehira, Formos. Trees rev. ed. 486. 1936.

Elaeagnus grandifolia Hay. Icon. Pl. Formos. **9**: 90. 1920. *Syn. nov.*
Elaeagnus nokoensis Hay. op. cit. 92. *f.* 32, *VII.*
Elaeagnus umbellata sensu Hay. in Journ. Coll. Sci. Tokyo **25** (19): 190. 1908 (Fl. Mont. Formos.), non Thunb.

A scandent shrub. Leaves coriaceous, lanceolate, 8–12 cm. long, 2–3 cm. broad, dark and glabrous above, silvery-brownish-lepidote beneath, acute at apex, rounded at base, entire, the costa impressed above, strongly raised beneath, the lateral veins 7 or 8 per side, indistinct, the reticulations obsolete; petioles 1–1.2 cm. long, brownish-lepidote. Flowers 1–3, axillary; pedicels to 1 cm. long including the elongated narrow ovary 3–4 mm. long; calyx campanulate, about 9 mm. long, silvery-lepidote without, the lobes oblong, acute, about 3 mm. long and 1.5 mm. broad.

Endemic to Formosa, in central mountain ranges.

Formosa: Between Sansyo-ambu and Yappitu, *S. Suzuki 5987* (NTY).

As noted above, Sasaki reduced this species to the synonymy of *E. thunbergii* Serv., but Kanehira seems to be correct in treating it as distinct. It is strongly characterized by the long lanceolate leaves. *Elaeagnus nokoensis* Hay. is included in the synonymy of *E. formosana* Nakai by Sasaki, but a photograph of the isotype (US) shows that Kanehira is right in treating it as a synonym of *E. morrisonensis*. *Elaeagnus grandifolia* Hay. was included in the synonymy of *E. thunbergii* by both Sasaki and Kanehira, but it seems to be referable instead to *E. morrisonensis*, except that the leaves are comparatively larger than in the type.

8. ELAEAGNUS FORMOSANA Nakai in Bot. Mag. Tokyo **30**: 74. 1916; Kanehira, Formos. Trees rev. ed. 483. *f. 444.* 1936.

Elaeagnus pungens sensu Matsum. & Hay. in Journ. Coll. Sci. Tokyo **22**: 356. 1906 (Enum. Pl. Formos.), non Thunb.

A scandent or suberect shrub; branches glabrous when mature, silvery-lepidote when young. Leaves coriaceous, broadly elliptic, 4–5 cm. long, 1.5–2.5 cm. broad, obtuse to acute at apex, obtuse to rounded at base, entire and usually revolute at margins, glabrous and shining or silvery-green when young above, silvery-lepidote beneath with scattered brown scales; petioles 4–12 cm. long, brownish-lepidote. Flowers fascicled, axillary; pedicels 5–6 mm. long; ovary oblong, 6–7 mm. long; calyx obtusely quadrangular, 3–4 mm. broad, the lobes about 3 mm. long, broadly triangular. Fruit oblong, about 1.3 cm. long and 6 mm. broad, slightly compressed, brown-lepidote.

Endemic, in thickets throughout the island at low to medium altitudes.

Formosa: From Sozan to Tansui, Taihoku-syu, *Zyo-En Ko 418* (NTY); Sozan, Taihoku, *E. H. Wilson 11230* (US).

This species is characterized by the thick coriaceous elliptic leaves, more or less obtuse at both ends.

The Destructiveness of Clitocybe Root Rot to Plantings of Casuarinas in Florida

ARTHUR S. RHOADS[1]

CONTENTS

USES AND ECONOMIC IMPORTANCE OF THE CASUARINAS

Of the numerous exotic plants, other than fruit trees, that occupy a useful place in Florida's horticulture, none are more widely used than certain species of *Casuarina*, natives of Australia and Oceania, commonly known as beefwood or shee-oak trees.[2] In Florida, however, where the horsetail beefwood (*C. equisetifolia*) was introduced prior to 1900, these trees invariably are termed Australian pines, with one species (*C. lepidophloia*) frequently designated by growers as New Zealand pine to distinguish it from the horsetail beefwood or common Australian pine.[3] The designation of these trees as pines, however, is misleading, since they are evergreen trees classified near the walnuts and hickories, although very unlike them. They are jointed plants suggesting the horsetails or scouring rushes (*Equisetum*) in structure, with the leaves

[1]Formerly Plant Pathologist of the Florida Agricultural Experiment Station, in charge of the Citrus Field Laboratory at Cocoa, in whose employ a large part of the work here reported was done. Grateful acknowledgment is made to the Division of Forest Pathology of the U. S. Department of Agriculture for their interest and support which have rendered it possible to complete this work and prepare the results for publication. Present location, William F. Clapp Laboratories, Duxbury, Mass.

[2]The "oak" figure of most of these woods is so well marked that in Australia they, with one exception, are called "oaks" or often "shee-oaks" on account of the peculiar sound produced by the wind when passing through the branches.

[3]The usage of common and scientific names conforms to that of the following authoritative source: Kersey, Harlan P., and William A. Dayton. Standardized plant names. 2nd edition. J. Horace McFarland Co., Harrisburg, Pa. 1942.

reduced to minute scales whorled at the nodes and decurrent on the internodes of the foliage branches, which the layman regards as needles.

The Casuarinas are probably adapted to a wider range of horticultural use and soils than any other tree in Florida. They are utilized extensively for windbreaks, street and roadside plantings, screen and ornamental plantings for quickly producing effects in home grounds, and hedges and other forms of topiary work. The horsetail beefwood, by reason of its tolerance of salt water and spray, is one of the most widely used trees in estates and landscaped grounds along the seacoast.

These versatile trees thrive on a wide range of soil types, including coastal beaches and land made by dredging sand and shell from under salt water, calcareous rocky land, marly soils, the muck of the Everglades, low hammock and high pine land, scrub land, and sand dunes. They are very tolerant of both dry and wet soil conditions but make their best growth in rich, moist soils. Under the latter conditions trees of lead pencil caliper may attain a height of 30 feet within 3 years from the time of planting. In places along the lower East Coast of Florida, both on natural land and that made by dredging, *C. equisetifolia* has become naturalized through self-seeding and is rapidly forming forests of considerable extent. By reason of their extreme rapidity of growth and adaptability to a diverse array of soil and soil moisture conditions, the Casuarinas have proved extremely useful and valuable for windbreak plantings in and about orchards of citrus and other subtropical fruits and are extensively used for this purpose in central and southern Florida.

Until 1940 *C. equisetifolia*, by reason of its earlier introduction and ready propagation from seed, was planted most extensively in the southern half of the State. Because of its marked susceptibility to cold and the fact that it makes a rather thin-topped tree to be particularly effective for windbreak purposes, the use of this species has been superceded of late years largely by the scalybark beefwood (*C. lepidophloia*). Except in those sections of the State that are exceptionally well protected from cold, the surviving plantings of *C. equisetifolia* were largely killed by the unusually severe freezes of January 28 and 29, 1940. The Cunningham beefwood (*C. cunninghamiana*) is also widely planted in the State and has proved hardy, even in the northern part, but is not so desirable for windbreaks. For this purpose, the scalybark beefwood (*C. lepidophloia*), which makes a compact, pyramidal crown and is fairly resistent to cold in central and southern Florida, has proved the most desirable of the several species of *Casuarina* available for use. This species is dioecious and, since apparently only the staminate form has become established in Florida, it must be propagated from root sprouts, which develop freely after the trees have grown a few years. In fact this species sprouts so freely, especially on rocky land, that plantings often become a nuisance. The swamp beefwood (*C. glauca*) also reproduces freely by means of root sprouts; the other species do not. Plantings of the latter species, as well as of *C. stricta* and *C. montana*, are as yet largely restricted to a few localities, and very small. There has been considerable confusion in the identity of the various species of *Casuarina* and, in former years, nurserymen often sold mixtures, so that windbreak plantings in some properties often contain two or three species. The confusion in species is further augmented by the fact that some are dioecious, while others are monoecious.

SUSCEPTIBILITY OF THE TREES TO CLITOCYBE ROOT ROT

Mushroom root rot caused by *Clitocybe tabescens* (Scop. ex Fr.) Bres., which the writer has found to be of widespread occurrence in Florida, has proved extremely destructive to plantings of Casuarinas in many parts of the State.[4] In situations where this disease is prevalent it usually develops early in the life of plantings, the initial infection of the trees taking place at one to several points in row plantings. The fungus spreads rapidly from tree to tree through the roots, especially where trees are planted closely. The trees die progressively from the original centers of infection, leaving gaps in the rows, which increase gradually in extent from year to year. When infection takes place more or less simultaneously at a number of points the rapid spread of the fungus soon works havoc with windbreak or roadside plantings, frequently decimating them in a short period or virtually destroying them within a few years. The large number of infected roots invariably left in the ground when dead or dying trees are removed provide additional centers of infection and thus increase the disease hazard. Replants set in places where infected roots have been removed incompletely invariably prove very short-lived, often dying within a year or two. Instances have been observed where the repeated loss of trees in hedge plantings has been so extensive that the entire hedge was dug up and replanted with something less susceptible. In many cases root rot has proved so destructive in windbreak plantings that, after one or two attempts at replanting, they are abandoned.

STUDIES ON MORTALITY FROM ROOT ROT IN PLANTINGS

Numerous instances of the dying of beefwood trees in ornamental, roadside and windbreak plantings in various parts of the State from Clitocybe root rot have been investigated during a period of 16 years. These have shown essentially the same features with respect to symptoms, attack at an early age, the inevitable spread of the fungus to adjacent trees and an extremely high mortality. The following cases are cited as examples of the destructiveness of the fungus in a few of the more extensive plantings in Brevard County, where the occurrence and spread of the disease have been studied most intensively.

Windbreak planting at Lotus, Merritt Island.—In October, 1930, a detailed inspection was made of extensive windbreak plantings in a citrus grove extending from the Banana River to Lake Wittfeld north of Lotus on Merritt Island, where extensive losses from Clitocybe root rot had been noted in 1928. The soil is a droughty sand which supported a growth of sand pine and scrubby oaks and other hardwoods prior to clearing. Trees were found to have died at irregular intervals throughout most of the planting and in a few places gaps occurred where as many as a dozen or more consecutive trees had died. Of 520 trees examined, 120 had been removed and 83 of those remaining were attacked. All but 4 of the latter were dead or dying. Thus, at the age of about 7 years, when the dead and dying trees ranged from 2–7 inches in diameter, the loss was nearly 40%.

[4]Aside from grass fires, cold, and occasionally lightning, root rot appears to be the only cause of death in these trees.

In addition, the death of a number of 18-month-old replants that had been set out in gaps where trees had died was noted. These trees ranged from ½–1 inch in diameter at the base. In one gap, 3 out of 6 of these replants were dead. Two others, which showed merely a slight yellowing of the foliage branches, were found to have the roots and root-crowns invaded by the Clitocybe root-rot fungus. Moreover, since the trees killed by root rot had been dug out 18 months before, the first 2 of the original trees on one side of these 6 replants and the first 4 on the other side were found to have succumbed to the disease. This left an extensive gap in the windbreak. The other replant exhibited no evidence of the fungus under the bark at the base but when examined again in May, 1931, the top had begun to die and the roots and root crown were found extensively invaded. These observations demonstrate the rapidity of spread of the fungus and the short life expectancy of replants when infected roots of trees which have succumbed to the disease are allowed to remain in the soil.

This planting was examined at intervals during the next decade, during which trees continued to die from root rot. By May, 1941, some of the largest surviving trees had attained a diameter of 15 inches at breast height but still proved susceptible to root rot, though a much longer period was required for trees of this size to die. Some trees of the scalybark beefwood were planted where trees of the horsetail beefwood had died and were removed but these also became attacked and killed at an early age. All the trees in the two rows originally planted across the grove at intervals back from the Banana River eventually died from root rot and the fungus fruited at the bases of many of these (Fig. 5A) and others. The original trees and replants through the central portion of the row along the front of this property also died. Most of the trees in the rows along the north and south sides of this grove likewise died. Thus, the progressive loss of trees from Clitocybe root rot over a period of about 15 years culminated in virtually complete failure of the plantings.

Indian River Bluff Subdivision at Melbourne.—Early in December, 1930, a detailed inspection was made of the extensive street plantings of horsetail beefwood trees that has been made about 4 years previously in Indian River Bluff subsivision north of Melbourne. The soil is a droughty sand and, prior to clearing, supported a mixture of slash pine and turkey oak, the latter remaining after the pine was logged. It was apparent that Clitocybe root rot was killing the beefwood trees in wholesale fashion. One of the developers of this subdivision stated that the mortality had been especially heavy during the past year and that, judging by the rapidity with which the trees were dying, there appeared little prospect of maintaining those remaining. A considerable number of the dead trees already had either been removed or had rotted and broken off. Forty-one of the dead trees remaining were attacked by the Clitocybe root-rot fungus, 27 having old dried clusters of sporophores at the bases. These trees mostly ranged from 2–4 inches in diameter at breast height. Six others, ranging from 4–5 inches in diameter, were still alive but from half to three-fourths defoliated and 4 of these exhibited more or less desiccated clusters of *C. tabescens*. Judging by the widespread occurrence of this fungus on the dead and

dying trees, it undoubtedly was responsible for the loss of those that had died and been removed previously. Additional evidence of the prevalence of this root-rot fungus was found in the yard of one residence, where it killed one plant in a young horsetail beefwood hedge and two in a Surinam cherry (*Eugenia uniflora*) hedge.

Indiatlantic Beach Subdivision at Melbourne.—In December, 1930, an inspection was made of the horsetail beefwood trees that had been planted about 4½–5 years previously along the streets and on individual lots in the Indiatlantic Beach subdivision between the Indian River and the Atlantic Ocean opposite Melbourne. The soil, being sand dune land, is very droughty. The vegetation, prior to clearly and grading, consisted chiefly of saw palmetto and a scrubby growth of oak and other hardwood trees. A considerable number of beefwood trees dead or dying from Clitocybe root rot were found scattered throughout a large part of the subdivision. The plantings nearest the ocean, where the growth had been dominantly saw palmetto, exhibited the greatest freedom from the disease. In places where trees had died and been removed a considerable number of replants were attacked. These beefwood plantings have continued to die from root rot. At the last inspection in April, 1941, a considerable number of trees still remained close to the ocean but elsewhere only scattered ones were left. Observations over more than a decade show that, while fire was responsible for the loss of some trees, Clitocybe root rot was the principal factor in the destruction of these plantings.

Windbreak plantings at Grant.—In December, 1930, another case of the extensive destruction of horsetail beefwood trees was observed in the extensive windbreak plantings of a large citrus grove between the Indian River and the Atlantic Ocean opposite Grant. The soil is Palm Beach sand with considerable shell in the subsoil. Prior to clearing, the land was covered with a dense hammock growth characterized by an abundance of live oak and other hardwoods and cabbage palmetto. Owing to the exposed situation of this grove it was thought necessary to plant windbreaks at frequent intervals. Consequently, in addition to the windbreak around the grove, a row of trees had been planted to every 4–6 rows of citrus trees throughout the grove, with cross rows at intervals. Most of the beefwood trees on the older part of the grove had been planted for 5 years but some for 7 years. Clitocybe root rot had ravaged parts of the windbreak plantings in the southern half of the grove during the two preceding years, though those in the northern half appeared free from this disease. The losses varied from a few consecutive trees to cases where a large number of consecutive trees had been killed, leaving long gaps in some of the rows. In the largest gap 45 consecutive trees had been attacked and all but 3 were dead. It was estimated by the grove manager that, of a total of about 40,000 trees planted, between 2,000 and 2,500 had died from this trouble. A large number of dead and dying trees were examined and these invariably showed an extensive development of the Clitocybe root-rot fungus between the bark and the wood and many also had either fresh or old clusters of sporophores of *C. tabescens* at the bases. The manager stated that a thousand replants had been set out a year previously to fill gaps where dead trees had been removed, and he estimated that

about 400 of these had died. All the replants examined were girdled by the root-rot fungus, although sporophores had developed on only a few. Many of the younger attacked trees had died so rapidly that they did not become defoliated appreciably but simply turned brown and dried up with the foliage branches remaining attached. Several living trees that merely showed a yellowing of the foliage branches on the lower portions of the crowns also were found to have the root systems attacked.

By April, 1941, the original citrus planting had been increased to 150 acres, with a windbreak maintained only around the periphery and between large blocks of grove. After maintaining these extensive windbreak plantings at frequent intervals throughout the grove for several years, it was found that they exerted a decided detrimental effect on the growth of the citrus trees and all those between the rows, totaling several thousand trees, were removed.

Property No. 1 at Artesia.—On September 1, 1931, an examination was made of a planting of horsetail beefwood trees on a property at Artesia, north of Cocoa Beach, where the sandy soil is characterized by droughtiness and the land, prior to clearing, was covered by a dense hammock with an abundance of live oak. These trees were planted in 1925 along the front of a property facing the county road and along each side of a lane leading into it. They had been dying for at least 3 years and those that had been dead longest had rotted and broken off. There were 93 trees in this planting originally. In 1931, only 46 trees were living and apparently healthy, 2 were living but partially defoliated as a result of girdling by root rot, and 8 were dead, 6 down and 31 missing. Thus, 47, or 50%, of these trees apparently succumbed to Clitocybe root rot. When this planting was next examined on April 30, 1941, this disease was continuing to attack the trees. Only 28 of the original 93 trees were still alive and all but 5 of those planted along the road had died.

Property No. 2 at Artesia.—Considerable mortality from Clitocybe root rot also was found in a more elaborate planting of horsetail beefwood alternating with coconut and royal palms on a property west of the preceding one. A large sum of money had been spent in developing this as an avocado grove and homesite. The trees were planted in 1925. The developer later sold the place and it has since been neglected. The ornamental planting consisted of 4 rows of beefwood trees with a row at right angles, in T-shaped fashion, at the west end bordering a salt marsh along the Banana River. This row and the two outer ones in the series had coconut palms alternating with the beefwood trees, while in the two inner rows royal palms alternated with them. The two inner rows were 30 feet apart, while the interval between these and the outer rows was but 20 feet. The trees were planted at intervals of 12½ feet in the rows, with the exception of a series of closely planted beefwood trees curving outward from the end of the two inner rows to the row along the west end to make an entrance formation (Fig. 1). These closely planted trees ranged from but a few inches to rarely more than a foot apart and were very small on account of crowding. On the right side all of the 34 crowded trees were living, while on the other, 18 consecutive trees were living and the remaining 19 dead. There was a total of 157 beefwood trees in this planting, including the two curved rows of closely set ones at the entrance. Of this number,

92 were living and apparently healthy, 2 were more or less yellow and defoliated from girdling by the root-rot fungus, 45 had been dead for varying lengths of time but were still standing, and 18 had been dead so long that they had rotted and broken off. Thus, 65, or 41%, of the beefwood trees in this mixed planting either had died or were practically dead from root rot. Gaps occurred where as many as 12, 10, 8 and 4 consecutive trees had died in the 4 rows, respectively (Fig. 1). Of

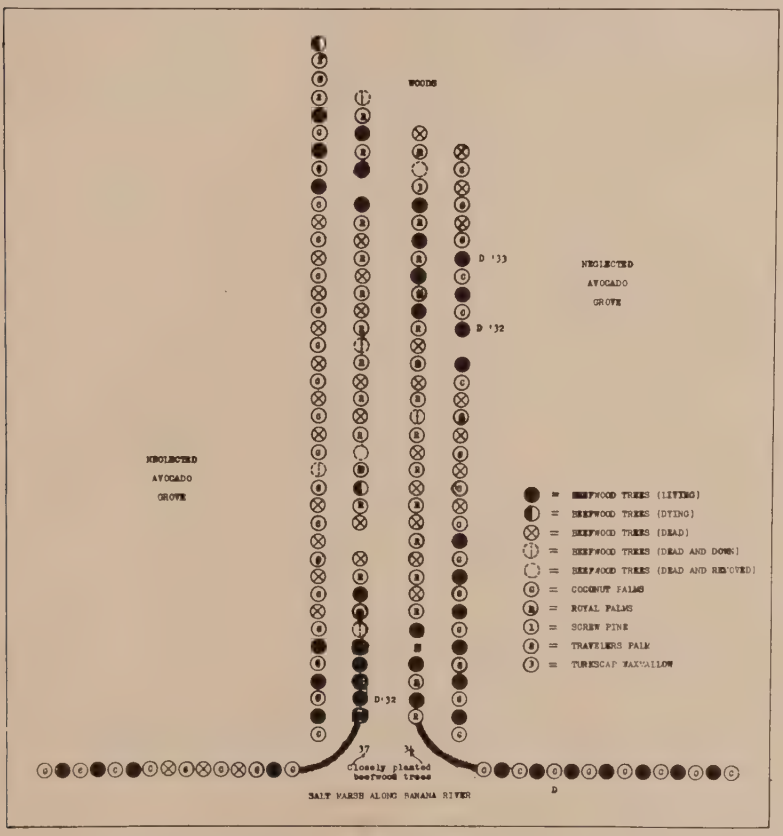

FIG. 1. Diagram of planting of horsetail beefwood trees alternating with coconut and royal palms, showing extreme susceptibility and high mortality of the former and complete resistance of the palms to Clitocybe root rot.

the palms, only one coconut was dead but did not die from root rot. In fact, it occurred in the right wing of the planting where no trees showed the disease at the time. Two royal palms in the second row from the north, one in the third row, and coconut palm in the fourth row were missing but were said to have been dug up for planting elsewhere. In subsequent inspections of this planting it was noted that two of the well-spaced beefwood trees were dead by August 5, 1932, and another by June, 1933. These are indicated by the symbols D'32 and D'33 (Fig. 1).

The final inspection of this planting was made April 30, 1941, by which time, owing to neglect for several years, a dense thicket of brush and vines had grown up. The root-rot fungus was found to have continued its inroads on the beefwood trees. Several additional trees had died at various times. Of the well-spaced trees the only ones remaining alive were the first three at the left in the left wing, the second in the second row on the left of the entrance, and the first two next to the entrance in the right-hand row. In the curved row of closely planted trees at the left the 18 trees still living in 1931 were dead. In the curved row of closely planted trees at the right, which were all living in 1931, the first 10 leading outward from the second row of trees from the right had been dead for some time and the next two were completely girdled by the fungus and dying. The twelfth and thirteenth trees had grown together at the base and the root-rot fungus was observed to have spread through the bark from the former to the latter. The other 21 trees in this row were still living and apparently free from the disease. The royal palms all appeared in good condition considering the neglect of the planting but the leaves of the coconut palms were severely injured by the freezes of January 28 and 29, 1940. The way in which the coconut and royal palms completely withstood the root-rot fungus when all the beefwood trees but 6 of the well-spaced ones and 21 of the small crowded ones interplanted with them succumbed, indicates a high degree of resistance for these palms. In fact, neither has thus far been found attacked by root rot at any point in the State.

Windbreak planting at Georgiana, Merritt Island.—On October 15, 1931, an inspection was made of an extensive windbreak planting of horsetail beefwood trees around three sides of a citrus grove on Norfolk fine sand at Georgiana, Merritt Island, about a year and a half previously. Since several of these young trees were found to have died from Clitocybe root rot at a very early age, the entire planting was plotted to depict the development and rate of spread of the disease. The row of trees along the north side of this grove extended from the east shore of the Indian River to the flats of the Banana River, a distance of nearly a half mile. The trees were numbered from 1 to 263. A citrus grove adjoined this windbreak on the north, extending from the Indian River to tree No. 55. From this point to tree No. 225 there adjoined cut-over slash pine woods with dense oak scrub, saw palmetto and, in places, muscadine grapevines. From this point to the corner of the grove near the grove near the Banana River an abandoned citrus grove adjoined, cultivation of this being resumed later. The fringe of scrub that had sprung up between the two groves from trees Nos. 225 to 250 was grubbed out during the summer of 1934. In mapping the planting it was noted that several trees had died from other causes than root rot shortly after planting. A few others were broken off in cultivating the grove.

In the third inspection of July 26, 1932, the 22 trees listed as dead or dying from root rot by February 15, 1932, together with 5 others that had died since then, were found to have been dug out and replaced by young trees on May 2. At this time the attacked trees ranged from ½–1 inch in diameter at breast height, nearly all being smaller than an inch. No additional replanting was attempted as the

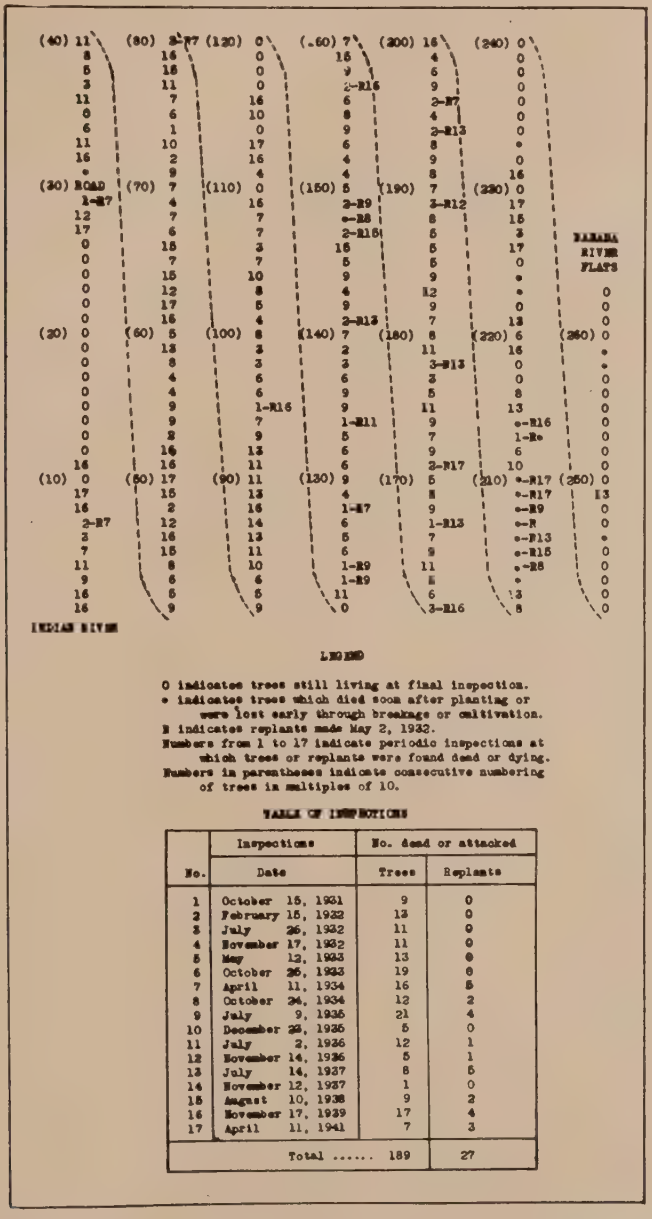

TABLE OF INSPECTIONS

No.	Inspections Date	No. dead or attacked Trees	Replants
1	October 15, 1931	9	0
2	February 15, 1932	13	0
3	July 26, 1932	11	0
4	November 17, 1932	11	0
5	May 12, 1933	13	0
6	October 26, 1933	19	0
7	April 11, 1934	16	5
8	October 24, 1934	12	2
9	July 9, 1935	21	4
10	December 23, 1935	5	0
11	July 2, 1936	12	1
12	November 14, 1936	5	1
13	July 14, 1937	8	5
14	November 12, 1937	1	0
15	August 10, 1938	9	2
16	November 17, 1939	17	4
17	April 11, 1941	7	3
	Total	189	27

FIG. 2. Diagram of horsetail beefwood windbreak planting nearly a half mile long, showing progressive spread of Clitocybe root rot from numerous centers of infection, beginning when the trees were less than 18 months old. By the end of a 10-year-period 189, or 77%, of the original trees and all but two of the 29 replants, which were destroyed in cultivating the grove, had died from this disease.

trees continued to die so rapidly that further effort to maintain the windbreak appeared useless. The order and rate of dying of the original trees and replants during the nearly 10-year-period covered by the inspections is depicted in Fig. 2. This shows the progressive spread of the fungus, beginning at a few centers when the planting was less than a year and a half old, and later at others. As the trees became older a longer time was required for them to die. At the end of the 10-year period the largest trees had attained a diameter of 10 inches. By this time the only living trees, with few exceptions, were those where citrus groves adjoined at the north and east ends of the windbreak, and, of these, trees Nos. 262 and 263 had become so choked by grapevines that they had died a short time previously. A high mortality occurred even in trees on land that had been cleared for several years where the blocks of grove adjoined. In the central portion of the windbreak, where scrub adjoined closely and, in places, seriously crowded the trees, practically all of them eventually succumbed to root rot. At one point in this scrub where shining sumac (*Rhus copallina*) shrubs occurred there was a progressive dying and loss of 5 of these. *C. tabescens* was isolated from the roots of one in 1936 and carried to fruiting. This fungus was isolated again in 1937 from another sumac and also from an infected scrub oak root found in digging up this sumac. In 1939, 3 other sumacs in the same vicinity were found to have died from root rot.

Of 245 of the original trees in this windbreak planting, after deducting 18 that were lost early from miscellaneous causes, 189, or 77%, died from Clitocybe root rot by the end of the 10-year-period. In addition, of the 29 replants made shortly before May 2, 1932, most of which had to endure heavy competition from the encroaching scrub, all but two, which were destroyed in cultivating the grove, died from root rot within a period of 9 years.

Another row of horsetail beefwood trees had been planted irregularly at the edge of a thicket along the shore of the Indian River, where some beakpod eucalyptus (*Eucalyptus multiflora*) trees and oleanders (*Nerium oleander*) had been planted previously among a natural growth of miscellaneous hardwoods with some live oak, saw palmetto and cabbage palmetto. Of the 81 beefwood trees here the first 17 from the windbreak on the north side died but the rest, with the exception of a group of 4 much farther south, remained free from root rot. A few of the eucalyptus trees also succumbed to root rot but none of the oleanders.

Still another row of horsetail beefwood trees had been planted irregularly at the edge of a thicket along the Banana River side of this grove, where oak trees did not occur. Several of these trees were cut down subsequently, quite a few became choked with vines and some killed by fire, but only one was observed to have died from root rot during the 10-year-period they were under observation.

Roadside planting at Georgiana, Merritt Island.—On December 23, 1935, a detailed inspection was made of the first 50 trees on the north end of a horsetail beefwood roadside planting made late in 1931 at Georgiana, where dying from Clitocybe root rot had been observed for some months, beginning with 3 trees noted April 24, 1934, when less than 3 years old. These trees were planted along the county road on a

partially cleared strip of oak scrub on Norfolk fine sand forming a high bluff along the Indian River. On the occasion of this inspection 27 of these trees had been dug out, laid to one side, and circles 4 feet in diameter grubbed preparatory to setting new trees. An examination of the root systems verified the fact that all had died from root rot.

FIG. 3. Portion of 10-year-old scalybark beefwood windbreak, showing tree largely defoliated from girdling by *Clitocybe* root rot in contrast with healthy trees on either side.

Three additional attacked trees had died since the removal of the others. The losses ranged from individual trees to groups of as many as 4 consecutive ones scattered throughout the planting, involving 60% by the time it was about 4 years old, at which time the trees averaged 3 inches in diameter. On July 2, 1936, 7 additional trees were observed to be dead or dying from root rot, and the balance of the first 46 trees on the north end of the planting died during the next few months. These were replaced by young trees of scalybark beefwood in 1937. One of these was observed to be dead from root rot on December 12, 1938. By April 11, 1941, when this planting was next inspected, 22 trees scattered throughout this second planting were already dead or dying from root rot. Thus, within the period of a scant 4 years 48% of this second planting of trees had succumbed to this destructive disease. The experience with plantings of beefwood trees on this and other properties shows that it is futile to make them in or adjacent to oak scrub.

SYMPTOMS OF THE DISEASE

The symptoms of Clitocybe root rot in beefwood trees vary somewhat according to the size of the trees and the rapidity of decline, young trees dying much more rapidly than older ones. The first symptom of the disease usually is a slight yellowing of the foliage branches, which develops first on the lower branches of the crown, on the side where the roots first become attacked. In the early stages of the disease this yellowing is so inconspicuous that it would not attract the attention of anyone not familiar with this disease.

With the progress of the disease this yellowing of the foliage branches becomes more pronounced and gradually progresses until it involves a large part of the crown.[5] This is followed by a gradual shedding of the worst affected foliage branches, resulting in the tree becoming characterized by a thin, pallid, sickly appearance and by an unusually heavy litter of foliage branches covering the ground. As a rule, attacked trees become two-thirds defoliated, and often completely so on the terminal portion of the crown, before death ensues (Fig. 3). However, in cases where the disease progresses with considerable rapidity in young trees, girdling may ensue so rapidly that the entire crown of a tree that appeared perfectly healthy may turn brown and die rapidly without the usual yellowing and shedding of the foliage branches.

The rapidity of girdling of attacked trees varies considerably according to the point at which the attack takes place, the rate of growth of the fungus and the size of the tree involved. While several months may be required for small trees attacked by the root-rot fungus to become completely girdled, medium-sized to large ones may require as much as two to three years, or even longer. In such delayed cases of dying the tree may develop new foliage branches following the shedding of a large portion, but these are characterized by being only about half the usual

[5]Yellowing from root rot should not be confused with a similar but more general yellowing or chlorosis occurring chiefly on trees on calcareous soils and resulting from a manganese deficiency. In severe cases of this particular trouble there is a marked reduction in the length of the foliage branches and a pronounced retarding of growth and stunting of the tree, which becomes thin-topped and scraggly.

length for the species. With the progress of the disease the foliage branches become quite yellow and progressively sparser from gradual shedding. When the girdling of the root crown is complete the sparse foliage branches remaining invariably show a distinct wilting and browning before the tree dies.

FIG. 4. Young horsetail beefwood tree with foliage branches yellow and top thin from attack by Clitocybe root rot. Bark peeled to show general infection of lower root system and spread of fungus upward around root crown and out on three lateral roots (one at rear) that were still green.

In the earliest discernible stage of the disease, when merely a slight yellowing of the lower portion of the crown develops on one side of the tree, it is rarely possible to detect the causal fungus by cutting into the bark at the base, since the mycelium usually has not progressed sufficiently to appear above the ground. However, by the time the yellowing of the foliage branches becomes general throughout the crown and they begin shedding it is usually possible to find the white to cream-colored sheets of mycelium developed between the bark and the wood, and also within the inner layers of the bark, at the base of the tree, at least on one side (Fig. 4). This may or may not be accompanied by a slightly sunken lesion resulting from the failure of the invaded tissues to keep pace in growth with those of the rest of the trunk. Such basal lesions usually are more apparent on young trees than on older ones with thicker bark and often develop first on one side before encircling the trunk. When such basal lesions develop the mycelium under the bark is found to be coextensive with them. By the time the crown of the tree becomes distinctly thin from shedding of the foliage branches the cutting test of the bark at the base usually shows that the mycelium has completely encircled it. This girdling frequently is accompanied by occasional cracking of the bark as it dies, at least in thin-barked trees. The basal lesions usually extend upward from a few inches to about a foot, and rarely as high as 2 or more feet, above the ground before attacked trees die. Their height frequently is greater on one side than on the other. It varies according to the size of the tree and the rapidity of death and usually is greater on good-sized trees than on small ones.

Clusters of sporophores of the causal fungus may or may not develop at the base of attacked trees before they die (Fig. 5). The production of these depends largely upon seasonal and soil moisture conditions. With favorable soil moisture conditions, they develop most frequently during the fall and early winter, from about the middle of September to the middle of December. As a rule but one or two clusters are developed during the year. In some instances the fungus may fruit when the trees show only a slight yellowing of the foliage branches.

After the death of attacked trees long cracks develop in the thin-barked *C. equisetifolia* and the bark gradually loosens and sheds in long strips. When this stage is reached the mycelium of the fungus between the bark and wood at the base of the tree above the ground dies as a result of desiccation.

The black, rounded or flattened, cortical and subterranean, shoestring-like rhizomorphs so frequently accompanying the closely related root-rot fungus, *Armillaria mellea* Vahl ex Fries, do not occur in the case of *C. tabescens*. Roots of trees attacked by the latter fungus, however, often exhibit a development of black, indurated xylostroma outgrowths extruded through longitudinal fissures in the bark. These extrusions have been reported to occur also in the case of the root rot caused by the well-known honey agaric (*A. mellea*) and have been regarded as rhizomorphs by some investigators. Their origin and anatomy have been discussed by the writer elsewhere (6).

Trees of *Casuarina lepidophloia* girdled by Clitocybe root rot invariably are stimulated to develop a more or less pronounced hypertrophy of the trunk immediately above the girdled portion. Other species of

FIG. 5. *Clitocybe tabescens* fruiting at bases of young beefwood trees dying from root rot. A. *Casuarina equisetifolia* with several young clusters of sporophores. B. *Casuarina cunninghamiana* with mature sporophores.

Casuarina and various other trees, particularly when young and thin-barked, often show a slight sunken or constricted effect where the wood accretion ceases on the basal portion girdled by the root-rot fungus, but merely exhibit a slight cracking of the bark at the point of callus formation above, and no particular tendency to hypertrophy. This hypertrophied effect in *C. lepidophloia* usually is further accentuated by the development of a pronounced callus formation at the lower limit to which the bark remains alive. This is accompanied by an abnormal thickening and blackening of the outer corky portion of the bark, which ruptures irregularly and is pushed outward and sometimes also upward (Fig. 6). The tree here illustrated shows the hypertrophy beginning at a point 17 inches high on one side and 24 inches on the other, with the bark at the point of callus formation extruded as much as 4 inches.

This trunk hypertrophy occurs invariably on trees of *C. lepidophloia* attacked by Clitocybe root rot but has never been observed in attacked trees of either *C. equisetifolia* or *C. cunninghamiana*. It can be used as a diagnostic feature to distinguish *C. lepidophloia* from the other two species in mixed plantings. In one citrus grove, however, *C. stricta* also was found to exhibit a similar tendency to hypertrophy when attacked.

ISOLATIONS OF THE CAUSAL FUNGUS

From 1924 to 1944 *C. tabescens* has been isolated from the roots of 156 trees, shrubs and vines, comprising 90 species, attacked by root rot in various parts of Florida. In view of the constancy with which this fungus was isolated from such a diverse array of plants attacked by root rot in various parts of the State, no particular attempt was made to make a large number of isolations from beefwood trees. However, from 1931 to 1942 the fungus was isolated from 20 dying or recently dead trees, including several that had been inoculated, as follows: 2 *C. cunninghamiana*, 7 *C. equisetifolia*, 2 *C. glauca*, 6 *C. lepidophloia*, 1 *C. montana*, and 2 *C. stricta*. With the exception of one each from Brooksville and Lake Alfred, the balance were from Cocoa and various points on Merritt Island. Most of these isolates were carried to the fruiting stage.

INOCULATION EXPERIMENTS

Various attempts to produce infection of trees with *C. tabescens* were made over a period of several years, beginning in 1931. The earlier attempts, which involved placing agar slants from large test tube cultures against roots, both injured and uninjured, of native and other trees growing in woods and vacant lots, potted plants in the greenhouse, and beefwood and citrus trees planted in buckets in a lathhouse, were all unsuccessful. The repeated failure to produce infections by the use of agar cultures as inoculum indicated that this method was ineffective, probably due to the short-lived nature of the inoculum when subject to destruction by various soil-inhabiting organisms and desiccation, and the great length of time required for the fungus to produce infection. All the writer's inoculation experiments in the open were, of necessity, carried on under extremely droughty conditions.

In June, 1936, inoculation of several horsetail beefwood trees planted on a thoroughly cleared and grubbed sandy lot adjoining the writer's

residence at Cocoa were made, using a modification of the above method. A good-sized root under each tree was cut off at some distance from the trunk, brushed free of sand, and a 1 x 8-inch test tube culture of the fungus pushed over the root until the cut end was well down into the agar. The space between the root and the mouth of the tube was then plugged with cotton and the soil replaced. This method naturally

FIG. 6. Pronounced hypertrophy and extrusion of bark in scalybark beefwood tree at margin of callus above basal portion girdled by Clitocybe root rot.

introduced contamination but it was thought that it might retard desiccation of the inoculum and favor infection. When these inoculations were examined in November, however, the inoculum was found to have dried up and deteriorated and the roots were not only free from infection but the cut ends had callused and developed new roots, together with marked hypertrophy of the lenticels, stimulated by the moist environment created.

In November, 1936, 7 other horsetail beefwood trees were inoculated by placing basidiospores from a fresh spore print of *C. tabescens* in incisions in the bark of the root crown, just under the ground, and provided with a mulch. No infection developed.

On November 19, 1936, two of a group of four well-spaced trees of *Casuarina lepidophloia* from 8–10 inches in diameter at breast height on the grounds of the Citrus Experiment Station at Lake Alfred were inoculated with lengths of roots from a tree of *C. equisetifolia* that had died shortly before from Clitocybe root rot at Georgiana, Merritt Island. The pieces of roots used as inoculum ranged from 1–2 inches in diameter and 10–12 inches long and had a good development of mycelium between the bark and the wood. These were placed in contact with two uninjured roots of each of two trees and secured by wire. The inoculations were marked by numbered cypress stakes. The two adjoining uninoculated trees were retained as controls.

These trees were revisited for examination a year later. On the first tree, where the inoculations were made on shallow roots, No. 1 produced no infection and No. 2 only an infected area of bark ½ inch long by ⅜ inch wide. Good infections were produced on the other tree, where the inoculations were made on deeper roots. In No. 3, where the inoculum was secured between two divisions of a forking root, respectively 1½ and ⅞ inches in diameter, the bark infection on the larger root involved a length of 9 inches and about a third of the circumference, while that on the smaller roots involved a length of 10 inches on one side, extending back past the point where the root forked. In No. 4 the bark infection extended a distance of 11 inches on one side and top and involved 2¾ inches of the circumference. As pointed out by the writer (2), this constitutes the first record of artificial transmission of this root-rot fungus, although pure cultures were not used.

The inoculations were examined again a year later, at which time the sandy soil was extremely dry. In No. 2, where a spot infection was produced after the lapse of a year, no further spread was noted. In No. 3 the lesions on the forking root were found to have increased to lengths of 15 and 21 inches, respectively, while in No. 4 the lesion had not increased appreciably in extent. Even at the end of May, 1939, these lesions showed no further increase and appeared to have become delimited and were callusing in places at the margin of the killed bark. In No. 3, however, a ⅜ inch lateral close to the point of inoculation was found to have been attacked during the past year and the terminal portion killed. This root showed mycelial development and xylostroma outgrowths characteristic of *C. tabescens* for a distance of 18 inches toward the trunk from the stake marking the inoculations. A portion of it was removed for making isolations and pure cultures of this fungus were secured, as reported previously (3).

Five years from the date of inoculation both Nos. 3 and 4 were found

to have spread extensively toward the tree along the infected roots, No. 4 having reached the base of the tree and involved not only the lower roots, which were dead, but extending in a narrow streak 4 feet high up one side, where the bark was killed.

On January 14, 1942, inoculations Nos. 1 and 2 were found to have produced slight local lesions finally where the inoculum was in contact with the roots, but these infections did not appear to have spread. However, the tree became attacked by root rot at the base in some undetermined way and the central one of the 3 trunk divisions was dead. The other inoculated tree showed no evidence of decline of the top, although it was extensively attacked, and the check trees showed no evidence of the disease.

In November, 1938, several trees of *C. equisetifolia* and *C. lepidophloia* planted on the cleared lot adjacent to the writer's residence at Cocoa were inoculated by placing in contact with uninjured roots cultures of *C. tabescens* isolated from various trees and grown on 4-inch lengths of oak stems about ¾ inch in diameter. The inoculum was placed a foot from the trunks, alternating trees of both species being retained as checks. The succeeding months were extremely dry and young citrus trees planted in alternate rows with the beefwood trees showed a pronounced wilting most of the time. Upon examining the inoculations early the following June, only 2 trees of *C. equisetifolia* showed infection (3). All the trees of this species were killed back severely by the freezes of January 28 and 29, 1940, but later put out shoots from the lower portions of the trunks. By the end of May, 1940, 5 of the inoculated trees and also 2 of the alternating control trees of *C. equisetifolia* were found completely girdled by Clitocybe root rot and dead. On another of these inoculated trees on which successful infection was noted the preceding year the fungus was found to have worked along the inoculated lateral root and spread into the bark at that side of the base of the tree. *C. tabescens* was isolated from the roots of each of the 5 inoculated trees, and also from 2 of the control trees, as reported previously (4).

In May, 1942, one of the inoculated trees of *C. lepidophloia* was found to be showing initial symptoms of decline on the lower branches. Upon exposure of the root system the mycelium of *C. tabescens* was found to have spread from the point of inoculation to the trunk and to have invaded and killed the bark ⅝ of the way around the base. It also had spread outward on the inoculated root for a distance of 36 inches on top and 40 inches on the bottom from the point of inoculation, the distal portion of the root still being green. The fungus also was found to have spread downward a short distance below the root crown and completely girdled another lateral root but apparently had not worked down below the root crown on the other roots. Both these lateral roots were 2½ inches in diameter near the trunk and the tree had attained a diameter of 8 inches at breast height. None of the trees of this species of *Casuarina* were injured particularly by the freezes of January, 1940. *C. tabescens* was isolated from the inoculated root of this tree and sporophores of the fungus developed in the original test tube isolates.

These successful inoculation experiments demonstrate that, even under the extremely droughty conditions prevailing, infection of trees

by the Clitocybe root-rot fungus can be effected through uninjured roots, both by the use of pieces of naturally infected roots and pure cultures of the fungus grown on a woody substratum.

Plakidas (1), who also was unable to infect Pineapple pear and tungoil trees with pure cultures of *C. tabescens* when grown on agar or other quickly perishable substratum, later reported the successful infection of two very young and two older Pineapple pear trees, using cultures of the fungus grown on blocks of wood as the inoculum, after first wounding the tissues at the points of inoculation. He stated that, so far as he was aware, this was the first time that infection with this fungus had been obtained from pure culture inoculations. Plakidas cited the writer's early unsuccessful attempts to infect trees with pure cultures on agar medium and noted his having reported the production of infection on the roots of two large beefwood trees, using as inoculum pieces of roots from another tree killed by Clitocybe root rot. However, he overlooked the fact that the writer (3) had reported the successful infection of 2 other beefwood trees with pure cultures of this fungus on lengths of oak stems as the inoculum.

The successful inoculation experiments with *C. tabescens* confirm observations made on various occasions when root systems of attacked trees were exposed for study of the mode and extent of spread of root-rot infection and demonstrate that, under favorable conditions, the fungus can attack living, uninjured roots. It is therefore apparent that *C. tabescens* is a virulent pathogen capable of attacking the most vigorously growing plants and transmitted chiefly by root contact.

COMPARATIVE SUSCEPTIBILITY OF THE DIFFERENT SPECIES OF CASUARINA TO CLITOCYBE ROOT ROT

All the different species of *Casuarina* that the writer has observed planted to any particular extent in Florida appear to be extremely susceptible to attack by *C. tabescens*, though only *C. equisetifolia*, *C. lepidophloia* and *C. cunninghamiana* have been planted sufficiently extensively to justify comparisons on this point. Moreover, plantings of the different species have been made on a diverse array of soil types and effective comparison of their relative susceptibility to root rot can be made only when similar aged plantings of the different species occur in the same situation. However, observations on the extensive destruction of plantings of the common *C. equisetifolia* at various points in Brevard County, in the vicinity of Fort Pierce, Lake Placid, and at numerous points in central and southern Florida clearly show that this species is extremely susceptible.

C. lepidophloia appears about as susceptible as *C. equisetifolia*, although some growers in Brevard County are of the opinion that it is much less so. However, investigation showed that those who hold this opinion frequently base it on trees planted on low hammock land, where root rot usually does not occur so extensively, and are unconsciously comparing such plantings with those of *C. equisetifolia* made on high, droughty land, where root rot is very prevalent. At the writer's former residence at Cocoa he has had to combat root rot on trees of *C. lepidophloia* for several years and his observations here and on numerous other properties clearly show that this species is also very

susceptible, at least when planted on land where the disease occurs frequently. This is bourne out by the 48% mortality recorded previously in a planting of this species on a partially cleared strip of oak scrub at Georgiana on Merritt Island, where a row of *C. equisetifolia* trees died previously at an early age.

C. cunninghamiana also has proved quite susceptible but no particularly heavy mortality has been noted in any of the many plantings that have been inspected at various points about the State over a period of several years. However, most of these have been relatively small ones. From the small percentage of trees of this species that have shown root rot in the numerous plantings examined it appears that this is the most resistant of the several species occurring in the State. The hardiness of this species commends it for planting in the northern section.

C. stricta has proved extremely susceptible in the few plantings observed where root rot occurred. An unusual opportunity to compare the susceptibility of this species with that of *C. lepidophloia* was afforded by finding both interplanted between many of the orange trees in a block of grove at Courtenay, Merritt Island. This land had been in grove for close to 11 years and was cleared about 10 years before planting to grove, being first used for a nursery. On April 10, 1941, when the comparison was made, the *C. stricta* trees were about 5 years old and those of *C. lepidophloia* about 6 years old. Out of 91 trees of *C. lepidophloia*, 9 were dead or dying, while out of 66 trees of *C. stricta*, 33 were dead or dying from Clitocybe root rot. Since these two species were mixed throughout the grove block and the trees of *C. stricta*, though younger by a year, suffered a much higher rate of mortality, this species appears to be much more susceptible than *C. lepidophloia*. This is indeed a startling degree of mortality for trees on land that has been cleared for many years. The occurrence of the disease was not localized in any particular area but was scattered through the entire block of grove. However, none of the orange trees, which were on sour orange stock, were attacked.

Both the little planted *C. glauca* and *C. montana* also have proved very susceptible to Clitocybe root rot, though no very large plantings of either have been found.

The total number of beefwood trees attacked by Clitocybe root rot that have been examined to date in Florida, tabulated by species, is as follows:

Casuarina cunninghamiana	149
" *equisetifolia*	1544[6]
" *glauca*	22
" *lepidophloia*	660
" *montana*	16
" *stricta*	86
" (species not determined)	20

[6] In addition to this count, it is estimated that between 2,000 and 2,500 trees and 400 out of 1,000 replants died in windbreak plantings of one citrus grove between the Indian River and the Atlantic Ocean opposite Grant, and that several hundred others have died in extensive roadside plantings in the vicinity of Fort Pierce, Lake Placid, and in numerous smaller plantings at various points in central and southern Florida.

INCIDENCE OF THE DISEASE IN RELATION TO TIMBERED LAND AND SOIL CONDITIONS

Clitocybe root rot has been found to have a widespread distribution throughout a large part of Florida, attacking native trees, tungoil trees, ornamental trees, shrubs and vines, and fruit trees, including bananas, citrus, guavas and other subtropical ones. In this State alone it has been found attacking 210 species of plants belonging to 137 genera and 59 families. The frequency with which beefwood plantings are attacked varies greatly in different localities, being greater on the higher, well-drained, light sandy soils where oak and other hardwood trees were prevalent prior to clearing or trees are planted in close proximity to oak scrub. Such soils are dominantly acid in reaction and subject to drought at frequent, and often for protracted, periods. Losses of beefwood trees from this disease, however, are by no means confined to newly cleared timbered lands but may occur even on land that has been cleared for periods ranging from 20 to 50 years. In some cases uncleared areas of oak scrub adjoined but in others merely citrus groves of considerable size adjoined. In view of this it appears that infections must occur at times in some way other than by root transmission.

Losses in plantings of beefwood trees occur much less frequently as a rule on low hammock soils, which, though also characterized by the prevalence of oak and other hardwoods prior to clearing, are heavier, of greater moisture content and usually neutral or alkaline in reaction, at least in the subsoil, and often are more or less closely underlaid by marl or shell. The disease is unknown in the extensive plantings of beefwood trees and areas of natural reproduction of the horsetail beefwood from Miami to Homestead on the lower East Coast. While oak trees occasionally are of frequent occurrence in this section, the soils of eastern Dade County, with few exceptions, are alkaline, being closely underlaid by oölitic limestone. Where this does not extend to the surface, it is covered by a thin mantle of sand, muck, peat or fresh-water marl. Clitocybe root rot appears to be absent also in plantings on the prairie type of soil where trees, particularly oaks, do not occur, as for example, the drainage districts west of Vero Beach and Fort Pierce, and the muck soils of the Everglades. It has not been found in the typical flatwoods sections of the State, where the dominant vegetation consists of pine trees and saw palmetto, and oak and other hardwood trees do not occur, though plantings of beefwood trees are rarely made on such soils. These trees also appear to remain free from root rot in plantings along the lower East and West Coasts where the land is a mixture of sand and shell and is essentially droughty in character, especially where oak trees are lacking or scarce. Those coastal plantings of horsetail beefwood trees on land made by dredging sand and shell from under salt water, as at Davis Island in Tampa Bay and Sarasota on the West Coast, and at Fort Pierce and Miami Beach on the East Coast, appear to be characterized by complete freedom from root rot.

TREATMENT OF ATTACKED TREES

It has been found possible to save, by careful surgical treatment, beefwood trees attacked by Clitocybe root rot, provided the disease

is located and treatment administered before the disease becomes too far advanced. Unfortunately, however, it makes such extensive inroads on attacked trees before marked symptoms appear that by the time their unhealthy condition becomes apparent it is generally too late to save them. This is particularly true of young trees. By the time the disease is observed, investigation usually reveals that a large part of the root system is infected and the bases more or less completely girdled.

Careful surgical treatment and aeration of the root crown have proved very effective in the treatment of beefwood trees attacked by root rot and a considerable number have been saved thereby. The procedure has been described in detail for citrus (6). While there occasionally has been a recurrence of the disease, in general, the results over a period of several years have proved very satisfactory. Surgical treatment is of practical value chiefly where trees are prized for ornamental purposes. It can not be regarded as practical in extensive windbreak plantings, especially on land where they are highly subject to infection and the disease starts more or less simultaneously from a number of centers.

The recovery of attacked trees that have lost a considerable part of their root systems or have been partially girdled by root rot often can be expedited materially by banking the soil to a height of a foot above the upper limit of the partial girdle to induce the development of a new root system from the callus formed at the margin of the living bark. Under favorable moisture conditions new roots start developing in several weeks and within a year or two should attain sufficient size to contribute materially to the support and recovery of the tree. The details and value of this method of providing the tree with a new root system as a means of combating root diseases has been described in detail by the writer (5).

SUMMARY

The uses and economic importance of the Casuarinas, or so-called Australian pines, for ornamental, roadside and windbreak plantings in Florida are discussed. Aside from the susceptibility of some species to cold, the most serious drawback to the extensive plantings of these trees in many parts of the State has proved to be their extreme susceptibility to mushroom root rot caused by *Clitocybe tabescens*, which is of widespread occurrence and often highly destructive.

Examples are given of the extreme destructiveness of this disease over a period of a decade in a number of properties where Casuarinas have been planted as windbreak or roadside trees. The 6 species planted more or less extensively have been found very susceptible to it but *C. cunninghamiana* appears less so than the others.

The symptoms of the disease are described and illustrated and the basal girdling caused by it has been found to stimulate a conspicuous hypertrophy of the trees that appears to be a characteristic feature of this disease in one or two species but not developing in others.

C. tabescens has been isolated repeatedly in various parts of the State from various species of *Casuarina*, and also from a large array of other

plants comprising native forest trees, shrubs and vines, tungoil trees, and fruit trees attacked by mushroom root rot.

The successful infection of Casuarinas, both through the use of naturally infected roots and pure cultures of the fungus grown on lengths of oak stems, when placed in contact with uninjured roots, is reported. In both cases the fungus was reisolated and carried to the point of fruiting.

C. tabescens has been shown to be a virulent pathogen capable of attacking uninjured roots of the most vigorously growing trees and spreading rapidly to adjacent ones, especially when closely planted, as in windbreaks. While the principal mode of transmission appears to be by root contact, the frequent development of the disease in young beefwood plantings on land cleared and planted to citrus groves for periods ranging from 20 to 50 years indicates that infection also occurs at times in some other way.

The incidence of Clitocybe root rot is by far the greatest on well-drained, light sandy soils that are dominantly acid in reaction and droughty in character and, prior to clearing, supported a growth of oak and other hardwood trees. In view of the fact that the disease usually is of infrequent occurrence on low hammock soils, which, though they too supported a growth of oak and other hardwoods prior to clearing, are of greater moisture content and usually neutral or alkaline in reaction, it appears that other factors besides diseased roots serving as centers of infection are involved in its development.

Clitocybe root rot is absent in the extensive plantings of beefwood trees and areas of natural reproduction of the horsetail beefwood from Miami to Homestead, where, though oaks often occur in places, the soils are alkaline, being closely underlaid by oölitic limestone. It appears to be absent also in plantings on the prairie types of soil and on the muck soils of the Everglades, where trees, particularly oaks, do not occur. It also appears to be absent in the typical flatwoods sections of the State, where the dominant tree is pine, and oak and other hardwoods do not occur. Coastal plantings on land that is a mixture of sand and shell, with oaks lacking or scarce, are generally free from root rot, especially when such land has been made by dredging material from under salt water.

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Invertase Activity of Penicillium italicum Wehmer

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INTRODUCTION

Much of the deterioration of fruits by fungi is affected by the activity of their enzymes. In fruits the typical carbohydrate, sucrose, is assimilated by fungi with the help of the enzyme invertase. Although studies on enzyme activity have been made in a number of fungi, further information on enzyme action in other fungi must be obtained to complete our understanding. A review of most of the literature on enzyme action of fungi was published earlier (Mehrotra 1949). The present work deals with the activity of invertase, both intracellular and extracellular, of *Penicillium italicum* Wehmer, the fungus causing citrus fruit decay during its two principal stages of growth, viz., 1) young vegetative stage, and 2) sporulating stage.

MATERIAL AND METHODS

The fungus was isolated from a decaying orange and as usual a single spore culture was obtained. It was identified as[1] *Penicillium italicum* Wehmer, and was grown in two different media, one of which was the natural medium, viz., orange juice, and the other a synthetic medium of the following composition: peptone, 2 gms.; KH_2PO_4, 1 gm.; KCl, 0.5 gm.; $MgSO_4$, 0.5 gm.; $FeSO_4$, 0.01 gm.; sucrose, 30 gms. The orange juice was steam sterilized while the synthetic medium was autoclaved at 15 lbs. pressure for 15 minutes.

A set of Petri-dishes containing 10 c.c. of the medium was prepared for each medium used. Thus two sets of Petri-dishes containing the two media were obtained. A heavy suspension of spores was sown in Petri-dishes and then they were incubated at 25° C. After 48 hours of growth the mass of germ tubes and young vegetative mycelium was filtered from one-half of each set of Petri-dishes. The two mats of young vegetative mycelium (viz., one from orange juice and the other from synthetic medium) thus collected were placed in muslin bags, washed well first in running tap water and afterwards in sterile distilled water to remove any trace of nutrients that might be present. The water from them was squeezed out as far as possible and the whole mycelial mats were dried *in vacuo* over calcium chloride in a desiccator. Each one of them was then finely powdered in a glass mortar and kept for determination of intracellular invertase activity of the fungus. The filtered medium in each case contained the secreted enzymes and was preserved with a few drops of toluene for the determination of the extracellular invertase. The other half of each set of Petri-dishes was filtered after 15 days by which time the fungus had sporulated profusely. The rest of the procedure was the same as with the first-half of each set of Petri-dishes. Thus from each medium, in all, two samples of mycelial mats (viz., one of young vegetative

[1]The fungus also gave a positive reaction to the ferric chloride test of Raistrick et al. (1931).

mycelium and the other of sporulating mycelium) and two of filtered medium containing the secreted (extracellular) enzymes were obtained.

Invertase activity was quantitatively determined as follows:

(a) for intracellular enzyme study: 20 c.c. of 0.2% cane sugar, 10 c.c. of citrate buffer of pH 4.5, 7 c.c. distilled water, 0.1 gm. finely powdered mycelial mat and 1 c.c. toluene.

(b) for extracellular enzyme study: 20 c.c. of 0.2% cane sugar, 10 c.c. of citrate buffer of pH 4.5, 5 c.c. distilled water, 2 c.c. filtered medium containing secreted (extracellular) enzyme and 1 c.c. toluene.

The flasks containing the above reaction mixture were kept at 20° C. for 7 days. The amount of reducing sugar formed was determined by Somogyi's method (1945). In each case control flasks containing the same reaction mixture but with inactivated enzyme (inactivated by boiling) were also kept and the difference between the control and the active enzyme was the measure of invertase activity.

OBSERVATIONS AND DISCUSSION

The results, as tabulated in the accompanying table, show that the fungus does not secrete the enzyme invertase into the liquid media used. It is well known that fungi either transform the nutrients into

TABLE.—*Figures given in mgs. of reducing sugar formed in 10 c.c. of the total digested volume of 37 c.c. containing 0.1 gm. of powdered fungus mycelium or 2 c.c. of extracellular enzyme solution.*

Enzyme preparation	Intracellular activity	Extracellular activity
Juice—(A)	13.24	.....
Juice—(B)	11.07	.....
Synthetic—(A)	14.32	.....
Synthetic—(B)	10.60	.....

Juice—(A): Young vegetative stage in orange juice.
Juice—(B): Sporulating stage in orange juice.
Synthetic—(A): Young vegetative stage in synthetic medium.
Synthetic—(B): Sporulating stage in synthetic medium.

assimilable form by secretion of enzymes into the medium, or absorb them if they are present in assimilable form. Once the food material reaches the cell sap it is digested there by the intra-cellular enzymes. Therefore if the food material is present in the assimilable form then the need of secreting extracellular enzymes is minimized. As is expected, some fungi have been reported to secrete invertase while others have not been able to do so. Emoto (1923) and Bhargava (1943) in some members of Saprolegniaceae, Bose and Sarkar (1937) in some polypores, Saksena and Jafri (1948) in some *Pythium* species, and Mehrotra (1949) in some Phytophthoras reported the secretion of invertase into the medium. On the other hand, Bhargava (1943), and Saksena and Bose (1944) noticed the absence of extracellular invertase in case of some other members of the Saprolegniaceae. From the results it appears that sucrose as such is absorbed by *Penicillium*

italicum Wehmer which assimilates it with the help of intracellular invertase. Hence the necessity of secreting the extracellular invertase does not arise.

At its two principal stages of growth, viz., (a) young vegetative stage and (b) sporulating stage, the invertase activity of the fungus shows a decline as the fungus in culture passes from the vegetative to the sporulating stage. Similar results were reported by Bose and Sarkar (1937) on the enzymic activity of a number of polypores.

There was little difference between the invertase activity of the fungus in the natural and synthetic media used.

SUMMARY

The invertase activity of *Penicillium italicum* Wehmer, isolated from an orange, was studied both in natural and synthetic media at its two principal stages of growth, viz., (1) young vegetative stage and (2) sporulating stage.

The results show that the fungus does not secrete invertase into the medium. It is concluded that sucrose as such is absorbed by the fungus and then assimilated with the help of intracellular invertase.

Invertase activity decreases from the young vegetative stage to the sporulating stage.

Little difference in invertase activity was noticed between the natural and synthetic media used.

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New Species of Idona from Mexico
(Homoptera: Cicadellidae)

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Idona was erected as a subgenus of *Empoasca* by DeLong in 1931 and *Empoasca minuenda* Ball was selected as the genotype. DeLong and Caldwell (1937) raised the group to generic status and removed all of the other North American species previously included in Idona except *minuenda*.

Young (1951) redefined the genus and in addition to *minuenda* has now placed in it *aperta* (Beamer 1943), *hyalina* (Beamer 1943), and *rubens* (Beamer 1934) from the southwestern United States; also he has included *sexmaculata* (DeLong 1923) from Puerto Rico and *hyalina* (Osborn 1928) from Bolivia. Seven new species of *Idona* have been examined in the collections of Mexican Typhlocybinae and are described in the following pages.

The types of all the new species except *eborolora* are in the DeLong collection. The types of that species are in the Snow Collection at the University of Kansas.

Idona usitata n. sp.

A pallid species resembling *minuenda* (Ball) in general form and appearance, but with distinctive male genital structures. Length 2.2 mm.

Color: A nearly uniform golden-yellow with the disk of the clavus and the costal margin of each elytron a darker, golden-tan.

Genitalia: Pygofer without hooks. The base of the aedeagus is rather short and cylindrical and bears a pair of long, heavy, caudally-directed, ventro-lateral processes which cross each other at their bases and turn sharply ventrad at their apices The phalicata arises from the base almost between the ventro-lateral processes.

Holotype male and male paratypes collected at Vejuco, G'ro., Sept. 3, 1930 (MF 1790) by J. Parra; male paratype El Mante, Tamaul., Oct. 26, 1930 (MF 1775), collected by A. Dampf.

Idona biforma n. sp.

A variable species which resembles strongly the variety *clavigera* (Ball) of *minuenda*, but with distinctive genitalia. Length 2.6 mm.

Color: There are two color forms which may be distinct species, although their male genitalia are similar. The first form with the head and pronotum of the males an immaculate golden-yellow; the females with a pair of round, black spots on the face between the eyes, a pair of median, longitudinal, triangular black spots on the anterior portion of the vertex, a small, black spot next each eye, and paired, large, black spots on the pronotum, one pair on its anterior margin and the other pair on its disk. The scutellum is golden-yellow with its basal angles, a pair of small, median spots, and its apex black in both sexes. The elytra are translucent gold with their transverse veins enbrowned and with a black spot on the disk and at the apex of each clavus and four similar spots on each corium.

In the second form, the sexual dimorphism is not as striking. Head and pronotum golden with paired, median, longitudinal, black vittae crossing the vertex and with spots on the pronotum similar to those of the first form. In the males, the vertex markings are obscured and the spots on the pronotum are small; in the females, the vittae are very distinct and the spots are large and may fuse to form longitudinal bands. The face of both sexes lacks the spots seen in females of the first form. The scutellum is golden with the basal angles, a pair of median spots, and the sides of the apex black. The elytra are translucent gold with the transverse vein enbrowned and with the black markings similar to those of the first form except that the clavi along the commissural margin anterior to the discal spot are black.

Genitalia: Pygofers, pygofer hooks, valve, plates, and styles similar to those of *minuenda*. Base of aedeagus short, thick, cylindrical; ventro-lateral processes of the base long, sharply-pointed, cylindrical; phalicata rather short, thick, arising from the base slightly above the ventro-lateral processes.

The first form represented by the holotype male, allotype female, male and female paratypes from Chilpancingo, G'ro., Oct. 25, 1941, collected by DeLong and Good; female paratypes, Tres Cumbres, D. F., Oct. 21, 1941 (K 52) and Puebla, Pue., Oct. 18, 1941, DeLong, Good, Caldwell, and Plummer. The second form represented by paratype males, 10 Km. N. of Cuernavaca, Mor., Dec. 28, 1949, R. H. Beamer; paratype females, Chilpancingo, G'ro., Oct. 25, 1941, DeLong and Good. The Chilpancingo paratypes in the Snow Museum.

Idona gigantica n. sp.

The largest of the species in the genus, resembling *minuenda* (Ball) in general form and appearance, but with distinctive coloration and genitalia. Length 3.2 mm.

Color: Vertex, face, and pronotum an immaculate cream. The scutellum cream with its apex black and, in the male, with a small longitudinal, black stripe on its disk. The elytra translucent gold with a small, round spot near the basal angles of the clavus, a large spot on the disk, and the apex of each clavus black; similar black spots near the base of each corium and on the proximal quarter and middle of each costal margin.

Genitalia: Pygofers with long, slender, sharply-pointed pygofers arising on their dorso-caudal angles and directed caudad. The apices of the pygofers produced into slightly, curved, hooks which are broad at their bases and taper to sharply-pointed apices. Aedeagus with parallel, slender, dorsally-directed, ventro-lateral processes which extend slightly above the apex of the phalicata. The phalicata is long, parallel-sides, and nearly truncate apically, and is curved dorsad.

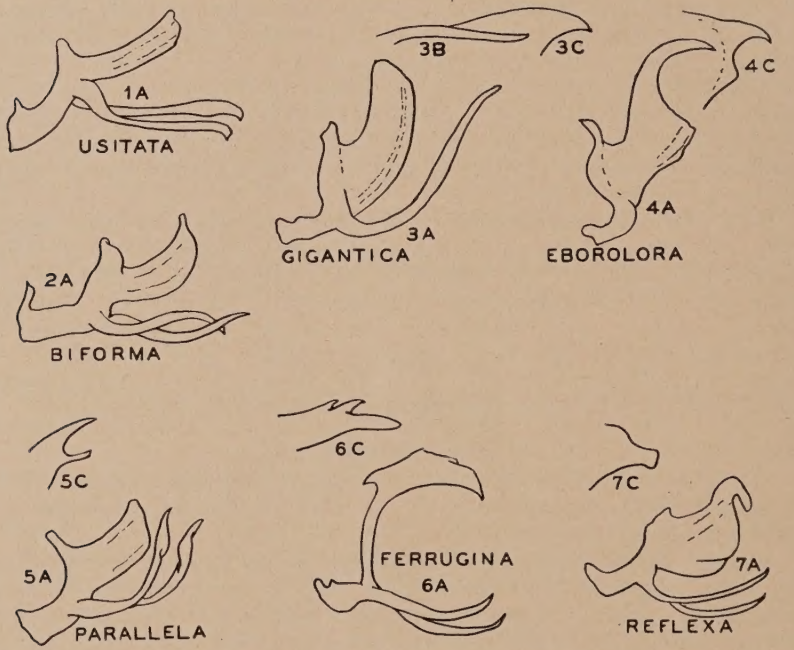
Holotype male collected at Cuernavaca, Mor., Oct. 21, 1941 (K 57) by DeLong, Good, Caldwell, and Plummer; paratype females collected at Mexico City, D. F., South in Canyon, Oct. 20, 1945 by D. M. DeLong.

Idona eborolora n. sp.

A highly colored species which resembles *minuenda* (Ball) in general form and appearance but which has distinctive coloration and genitalia. Length 2.6 mm.

Color: Vertex golden with its anterior margin broadly bordered

with ivory and with a pair of round, black spots near its apex. Face cream with its dorsal portion, genae, and lora ivory; immaculate in the male and with a small, black spot near the anterior corner of each eye and a larger, black spot just below each antenna in the female. The pronotum is translucent white with irregular spots of ivory and orange along its anterior margin and with paired, lateral and medial, round black spots on the anterior portion of the disk. A black spot is located on each side of the pronotum. The scutellum is cream with the basal angles and a median, longitudinal stripe orange and with the apex



FIGS. 1–7. 1a—*Idona usitata*—lateral view of aedeagus. 2a—*I. biforma*—lateral view of aedeagus. 3a—*I. gigantica*—lateral view of aedeagus; 3b—lateral pygofer hook; 3c—apex of pygofer. 4a—*I. eborolora*—lateral view of aedeagus; 4c—apex of pygofer. 5a—*I. parallela*—lateral view of aedeagus; 5c—apex of pygofer. 6a—*I. ferrugina*—lateral view of aedeagus; 6c—apex of pygofer. 7a—*I. reflexa*—lateral view of aedeagus; 7c—apex of pygofer.

and a spot on the middle of each lateral margin black. The elytra are translucent white with the transverse veins enbrowned and with a round black spot on the middle of the commissural suture, at the base of each corium, on the proximal quarter, and on the distal quarter of each costal margin. The disk of each clavus is light-orange, the apex of each clavus is black, and four, long, anastomosing, dark-orange spots are located on the disk of each corium. A pair of the females of the series, perhaps teneral forms, have the markings darker and larger, a black spot on the posterior margin of the vertex and on the disk of the pronotum. The anteclypeus and apex of the postclypeus are black.

Genitalia: Similar to *minuenda* in pattern, but the pygofers are without a hook; the apices of the pygofers produced into caudally-directed, C-shaped processes, the dorsal arms of which are much longer

and broader than the ventral arms. The aedeagus is without ventro-lateral processes, its dorsal processes are long and pointed; the phalicata is broad, cylindrical, abruptly reduced in width at its center, the apical process thus formed is bent nearly caudal near its center and tapers to a sharply-pointed apex.

Holotype male, allotype female, and female paratypes collected at Jacala, Hil., Jan. 2, 1950 by R. H. Beamer. Types in the Snow collection. Paratype in the DeLong collection.

Idona parallela n. sp.

Resembling *eborolora* in general form and appearance, but with distinctive coloration and genitalia. Length 2.2 mm.

Color: The vertex is cream with paired, anterior and posterior, medial, black spots on its disk; the spots joined in the specimen at hand by an irregular, brown, medial band. The face cream with a pair of small spots next to the anterior corner of each eye and a spot below each antenna black. The pronotum is cream with the central portion of its disk and spots along its anterior margin cream; with paired anterior and posterior, and paired lateral spots on its disk black. The scutellum is cream with its basal angles and a medial spot orange, and with the tip of its apex black. The elytra are translucent white with the transverse vein enbrowned and with black spots on the commissural margin and apex of each clavus. Black spots are located at the base, proximal quarter, and middle of each costal margin. The base and disk of each clavus are marked with orange spots. Four orange spots, one near the base, one near the center, and a pair near the transverse veins, are located on each corium.

Genitalia: Pygofers without hooks; their apices produced into small, C-shaped, medially-directed processes. The base of the aedeagus is short, broad, and cylindrical with the ventro-lateral processes rather heavy and strongly curved dorsad and with the phalicata rather short, thick, and nearly parallel sided.

Holotype male collected at Iguala, G'ro., Sept. 11, 1939 by D. M. DeLong.

Idona ferrugina n. sp.

This and the following species, while they resemble *minuenda* in venation and general form, differ from the rest of *Idona* in having a longer head, second apical cell of the elytron pedunculate, and in having the male plates short and broad. Length 2.5 mm.

Vertex with its median length about as long as its basal width between the eyes; second apical cell pedunculate.

Color: The vertex is ivory with a short, transverse, black line on the anterior margin at its apex. There is a pair of black spots at its apex and a similar spot next to each eye and one on the middle of its posterior margin. The dorsal portion of the postclypeus is cream with its ventral half dark brown and with the anteclypeus dark brown bordered with ivory; the genae and lora are dark brown with a horizontal band across the genae and the portion bordering each eye ivory. The pronotum is ivory with the central portion of its disk light brown and with four spots on its disk and anastamosing spots along its anterior margin brown. The sides of the thorax are black crossed by a broad, longitudinal ivory line. The elytra are translucent with the clavi and disks

of the coria flecked and mottled with reddish brown; the major portion of the costal margin and apical cells brown; and a costal plaque yellow.

Genitalia: The plate is broad, being widest at its middle; the lateral spine-bearing tubercule remote from the base of the plate; apex angulate. Pygofers narrowing distally to a spine-like, mesally-directed apex which in the holotype bears a pair of short, dorsal spines, these are absent in the paratype males. The ventro-lateral processes of the base arise near its proximal end and are rather heavy; the base above the ventro-lateral processes is erect, long, and very slender; the phalicata is a short, broad tube.

Holotype male, allotype female, male and female paratypes collected at Chilpancingo, G'ro., Oct. 25, 1941 by DeLong and Good.

Idona reflexa n. sp.

Resembling *ferrugina* in general form and appearance, but lighter color and with unique male genital structures. Length 2.2 mm.

Color: The vertex is ivory with its basal half light tan and with a pair of large spots at its apex and a smaller spot on the middle of its posterior margin black. The face is tan with its genae and lora ivory and with a black spot next to the anterior angle of each eye and a similar spot below each antenna. The pronotum is tan with four spots on its disk and the central portion of its disk black. The scutellum is cream with its basal angles tan, and with its apex and a spot on the middle of each lateral margin black. The disks of the clavi and coria are mottled with orange, the apical cells are enbrowned, and there is a black spot on the apex of each clavus, a spot on each humeral angle, and a pair of spots on the costal margin.

Genitalia: The external male genitalia are similar to those of ferrugina. The apices of the pygofers terminate in blunt, mesally-directed processes. The apices of the styles are sinuate. The ventro-lateral processes of the base are long and slender; the dorsal processes are small knobs; the phalicata is broad, its caudal margin is rounded, and it is extended at its apex into a thin, curved apical process.

Holotype male collected at Mazatlan, Chiapus, Nov. 12, 1932 (MF 2757) by Dr. Dampf; paratype females collected at Orizaba, V. C., Oct. 17, 1941 (K 28) by DeLong, Good, Caldwell, and Plummer.

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